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(71) Applicant (for all designated States except US): APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. [NL/NL]; Pietermaai 15, Curacao (AN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): IBBERSON, Mark [GB/CH]; Chemin Planet 41, CH-1188 Gimel (CH). POWER, Christine [GB/FR]; Rue des Jonquilles 10, F-01710 Thoiry (FR). FRAUENSCHUH, Achim [DE/CH]; Chemin de Voirets 1, CH-1228 Plan-les-Ouates (CH).

(74) Agent: SERONO INTERNATIONAL SA INTELLECTUAL PROPERTY; Chemin des Aulx 12, CH-1228 Plan-les-Ouates (CH).

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(54) Title: NOVEL CHEMOKINE-LIKE POLYPEPTIDES

(57) Abstract: The present invention discloses open reading frames (ORFs) in human genome encoding for novel chemokine-like polypeptides, and reagents related thereto including variants, mutants and fragments of said polypeptides, as well as ligands and antagonists directed against them. The invention provides methods for identifying and making these molecules, for preparing pharmaceutical compositions containing them, and for using them in the diagnosis, prevention and treatment of diseases.

WO 2004/031233 A2

- 1 -

NOVEL CHEMOKINE-LIKE POLYPEPTIDES

FIELD OF THE INVENTION

The present invention relates to nucleic acid sequences identified in human genome as encoding for novel polypeptides, more specifically for chemokine-like polypeptides.

BACKGROUND OF THE INVENTION

The mammalian immune response is based on a series of complex, network-like interactions involving cellular components (such as lymphocytes or granulocytes) and soluble proteins, capable of modulating cellular activities (movement, proliferation, differentiation, etc.). Thus, there is considerable interest in the isolation and characterization of cell modulating factors, with the purpose of providing significant advancements in the diagnosis, prevention, and therapy of human disorders, in particular the ones associated to the immune system.

Chemokines are amongst these soluble proteins, since they are involved in the directional migration and activation of cells. This superfamily of small (70-130 amino acids), secreted, heparin-binding, pro-inflammatory proteins is known especially for the role in the extravasation of leukocytes from the blood to tissue localizations needing the recruitment of these cells (Baggiolini M et al., 1997; Yoshie OF et al., 2001; Fernandez EJ and Lolis E, 2002).

Chemokines are not only functionally related but also structurally related, since they all contain a central region in which conserved Cysteines form intramolecular bonds. In particular, the number and the position of the most N-terminal of these conserved Cysteines in the mature polypeptides is the basic criteria for the generally

- 2 -

recognized classification of chemokines, essentially divided between chemokines having a single or adjacent Cysteines (C-C chemokines), or chemokines having two Cysteines separated by 1-3 amino acids (C-X-C chemokines).

A series of membrane receptors, all heptahelical G-protein coupled receptors, are 5 the binding partners that allow chemokines to exert their biological activity on the target cells. The physiological effects of chemokines result from a complex and integrated system of concurrent interactions. Different cells can present specific combinations of receptors according to their state and/or type. Moreover, chemokine receptors often have overlapping ligand specificity, so that a single receptor can bind different 10 chemokines, as well a single chemokine can bind different receptors, still at high affinity.

Usually chemokines are produced at the site of an injury, inflammation, or other tissue alteration, and exert their activity in a paracrine or autocrine fashion. However, 15 cell-type specific migration and activation in inflammatory and immune processes is not the sole activity of chemokines. Other physiological activities, such as hematopoiesis or angiogenesis, and pathological conditions, such as metastasis, transplant rejection, Alzheimer's disease or atherosclerosis, appear to be regulated by, at least, some of these proteins. In fact, chemokines and/or their receptors have been found considerably over-expressed and/or activated in several animal models or clinical 20 samples (Haskell CA et al., 2002; Lucas AD and Greaves DR, 2001; Frederick MJ and Clayman GL, 2001; Godessart N and Kunkel SL, 2001; Reape TJ and Groot PH, 1999).

There are potential drawbacks in using chemokines as therapeutic agents (tendency to aggregate and promiscuous binding, in particular), but molecules having 25 antagonistic properties against chemokines are widely considered as offering valuable

- 3 -

opportunities for therapeutic intervention in disorders associated to excessive chemokine activities. The inhibition of specific chemokines and their receptors is considered a solution for preventing undesirable or uncontrolled cellular processes, such as recruitment or activation (Baggiolini M, 2001; Proudfoot A, 2000 ; Rossi DF and 5 Zlotnik A, 2000).

The extensive sequencing programs and bioinformatics have made available a large amount of tools and information on human genome and physiology (Quinn-Senger KE et al.; 2002; Browne MJ, 2000). Such technologies were also used for discovering novel chemokines and receptors possibly providing new and useful 10 therapeutic molecules and targets. Initially, chemokines genes were regularly mapped on chromosomes 4 and 17, in gene-rich areas of human genome (Nomiyama H et al., 2001), but the literature provides various approaches for characterizing novel 15 chemokines by comparing the tissue-distribution of transcripts. Chemokines are usually expressed in lymphoid and other tissues but novel chemokines can have specific expression patterns and can be mapped to chromosomal loci different from the traditional gene clusters (WO 02/70706; Wells TN and Peitsch MC, 2000; Chantry DF et al., 1998; Rossi D et al., 1997).

Novel chemokines have been identified by applying strict homology criteria to known chemokines. However, since the actual content in polypeptide-encoding 20 sequence in human genome for chemokines (as for any other protein family) is still unknown, the possibility still exists to identify DNA sequence encoding for polypeptides having chemotactic activities by applying alternative criteria in the analysis of Open Reading Frames (ORFs, that is, DNA sequences containing consecutive coding triplets of nucleotides, not interrupted by a termination codon and that can be potentially 25 translated in a polypeptide) present in human genome.

- 4 -

SUMMARY OF THE INVENTION

The invention is based upon the identification of Open Reading Frames (ORFs) in human genome encoding novel chemokine-like polypeptides.

Accordingly, the invention provides isolated polypeptides having the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16, and their mature forms, variants, and fragments, as polypeptides having chemotactic activity. The invention includes also the nucleic acids encoding them, vectors containing such nucleic acids, and cell containing these vectors or nucleic acids, as well as other related reagents such as fusion proteins, ligands, and antagonists.

The invention provides methods for identifying and making these molecules, for preparing pharmaceutical compositions containing them, and for using them in the diagnosis, prevention and treatment of diseases.

DESCRIPTION OF THE FIGURES

Figure 1: alignment of the ORF contained in the DNA sequence GNSQ_1754 (SEQ ID NO: 1) with the protein sequence p1754 (SEQ ID NO: 2). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated with §. The arrows indicate the position of the primers CL_1754_5 (forward) and CL_1754_3 (reverse) in the ORF sequence.

Figure 2: alignment of the ORF contained in the DNA sequence GNSQ_0711 (SEQ ID NO: 3) with the protein sequence p0711 (SEQ ID NO: 4). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated

- 5 -

with §. The arrows indicate the position of the primers CL_0711_5 (forward) and CL_0711_3 (reverse) in the ORF sequence.

Figure 3: alignment of the ORF contained in the DNA sequence GNSQ_2882 (SEQ ID NO: 5) with the protein sequence p2882 (SEQ ID NO: 6). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated with §.

Figure 4: alignment of the ORF contained in the DNA sequence GNSQ_4711 (SEQ ID NO: 7) with the protein sequence p4711 (SEQ ID NO: 8). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated with §.

Figure 5: alignment of the ORF contained in the DNA sequence GNSQ_4320 (SEQ ID NO: 9) with the protein sequence p4320 (SEQ ID NO: 10). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated with §. The arrows indicate the position of the primers CL_4320_5 (forward) and CL_4320_3 (reverse) in the ORF sequence.

Figure 6: alignment of the ORF contained in the DNA sequence GNSQ_5008 (SEQ ID NO: 11) with the protein sequence p5008 (SEQ ID NO: 12). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated with §. The arrows indicate the position of the primers CL_5008_5 (forward) and CL_5008_3 (reverse) in the ORF sequence.

- 6 -

Figure 7: alignment of the ORF contained in the DNA sequence GNSQ_0210 (SEQ ID NO: 13) with the protein sequence p0210 (SEQ ID NO: 14). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated with §. The arrows indicate the position of the primers CL_0210_5 (forward) and CL_0210_3 (reverse) in the ORF sequence.

5 Figure 8: alignment of the ORF contained in the DNA sequence GNSQ_4922 (SEQ ID NO: 15) with the protein sequence p4922 (SEQ ID NO: 16). The predicted N-terminal signal sequence is boxed. The predicted C-terminal alpha helix is underlined. The codons matching the original selection criteria are indicated with §. The arrows indicate the position of the primers CL_4922_5 (forward) and CL_4922_3 (reverse) in the ORF sequence.

10 Figure 9: alignment of human CXCL chemokines with the CXC chemokine-like protein sequences of the invention p1754 (SEQ ID NO: 2), p0711 (SEQ ID NO: 4), p2882 (SEQ ID NO: 6), p0210 (SEQ ID NO: 14), and p4922 (SEQ ID NO: 16). The following human CXCL chemokines have been considered: CXCL1 (SWISSPROT Acc. N° P09341), CXCL2 (SWISSPROT Acc. N° P19875), CXCL3 (SWISSPROT Acc. N° NP_002081), CXCL4 (SWISSPROT Acc. N° NP_002610), CXCL5 (SWISSPROT Acc. N° P42830), CXCL6 (SWISSPROT Acc. N° P80162), CXCL7 (SWISSPROT Acc. N° P02775), CXCL8 (SWISSPROT Acc. N° P10145), CXCL9 (SWISSPROT Acc. N° Q07325), CXCL10 (SWISSPROT Acc. N° P02778), CXCL11 (SWISSPROT Acc. N° Q14625). The protein sequences are divided according to the structure of the three main regions: the N-terminal region (containing the signal sequence), the central Cys-rich region (containing the conserved Cysteines matching the

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original selection criteria and indicated with §), and the C-terminal region (containing the predicted alpha helix).

Figure 10: alignment of human CCL chemokines with the CXC chemokine-like protein sequences of the invention p4711 (SEQ ID NO: 8), p4320 (SEQ ID NO: 10), and GNSQ_5008 (SEQ ID NO: 12). The following human CCL chemokines have been considered: CCL1 (SWISSPROT Acc. N° P22362), CCL2 (SWISSPROT Acc. N° P13500), CCL3 (SWISSPROT Acc. N° P10147), CCL4 (SWISSPROT Acc. N° P13236), CCL5 (SWISSPROT Acc. N° P13501), CCL7 (SWISSPROT Acc. N° P80098), CCL8 (SWISSPROT Acc. N° P80075). The protein sequences are divided according to the structure of the three main regions: the N-terminal region (containing the signal sequence), the central Cys-rich region (containing the conserved Cysteines matching the original selection criteria and indicated with §), and the C-terminal region (containing the predicted alpha helix).

Figure 11: Map of the pEAK12d expression vector.

DETAILED DESCRIPTION OF THE INVENTION

The main object of the present invention is to provide novel, isolated polypeptides having chemotactic activity selected from the group consisting of:

- the amino acid sequences SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16;
- the mature form of the polypeptides SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16;
- the polypeptides comprising the Cysteine-rich region of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16, as indicated in fig. 9 and 10;
- the active variant of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 wherein any amino acid specified in the chosen sequence is

- 8 -

non-conservatively substituted, provided that no more than 15% of the amino acid residues in the sequence are so changed;

e) the active fragments, precursors, salts, or derivatives of the amino acid sequence's given in a) to d).

5 The novel polypeptides p1754 (SEQ ID NO: 2), p0711 (SEQ ID NO: 4), p2882 (SEQ ID NO: 6), p4711 (SEQ ID NO: 8), p4320 (SEQ ID NO: 10), p5008 (SEQ ID NO: 12), p0210 (SEQ ID NO: 14), and p4922 (SEQ ID NO: 16) were identified on the basis of a consensus sequence for human chemokines in which the number and the position of selected amino acids (initial methionine, cysteines, and hydrophobic residues) are 10 defined for protein sequence having length comparable to known chemokines.

The totality of amino acid sequences obtained by translating the known ORFs in the human genome were challenged using this consensus sequence, and the positive hits were further screened for the presence of predicted specific structural and functional "signatures" (a N-terminal signal sequence and a C-terminal alpha helix), 15 and finally selected by comparing sequence features with known chemokines. Therefore, the novel polypeptides of the invention can be predicted to have chemotactic activities.

The terms "active" and "activity" refer to the chemotactic-like properties predicted for the chemokine-like amino acid sequences SEQ ID NO: 2, 4, 6, 8, 10, 12 14, or 16 in 20 the present patent application.

Protein sequences having the indicated number of non-conservative substitutions can be identified using commonly available bioinformatic tools (Mulder NJ and Apweiler R, 2002; Rehm BH, 2001).

In addition to such sequences, a series of polypeptides forms part of the 25 disclosure of the invention. Being chemokines known to go through maturation

- 9 -

processes including the proteolytic removal of N-terminal sequences (by signal peptidases and other proteolytic enzymes), the present patent application also claim the mature form of the polypeptides SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16. As mature form is intended any polypeptide showing chemotactic activity and resulting from *in vivo* (by the expressing cells or animals) or *in vitro* (by modifying the purified polypeptides with specific enzymes) post-translational maturation processes. Mature forms of chemokines resulting from C-terminal processing are also known (Ehlert JE et al., 1998). Other alternative mature forms can also result from the addition of chemical groups such as sugars or phosphates.

10 A further group of polypeptides of the invention are the polypeptides comprising the Cysteine-rich region of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16, as indicated in fig. 9 and 10, since the central Cysteine-rich region contains the essential structural and functional groups of chemokines.

15 Other claimed polypeptides are the active variants of the amino acid sequences given by SEQ ID NO: 2, 4, 6, 8, 10, 12 14, or 16 wherein any amino acid specified in the chosen sequence is non-conservatively substituted, provided that no more than 15% of the amino acid residues in the sequence are so changed. The indicated percentage has to be measured over the novel amino acid sequences disclosed in figures 1-8, and in particular over a segment of at least 40 amino acids containing the 20 Cysteine-rich regions as indicated in figures 9 and 10.

In accordance with the present invention, any substitution should be preferably a "conservative" or "safe" substitution, which is commonly defined a substitution introducing an amino acids having sufficiently similar chemical properties (e.g. a basic, positively charged amino acid should be replaced by another basic, positively charged

amino acid), in order to preserve the structure and the biological function of the molecule.

The literature provide many models on which the selection of conservative amino acids substitutions can be performed on the basis of statistical and physico-chemical 5 studies on the sequence and/or the structure of proteins (Rogov SI and Nekrasov AN, 2001). Protein design experiments have shown that the use of specific subsets of amino acids can produce foldable and active proteins, helping in the classification of amino acid "synonymous" substitutions which can be more easily accommodated in protein structure, and which can be used to detect functional and structural homologs 10 and paralogs (Murphy LR et al., 2000). The groups of synonymous amino acids and the groups of more preferred synonymous amino acids are shown in Table I.

Active variants having comparable, or even improved, activity with respect of corresponding chemokines may result from conventional mutagenesis technique of the encoding DNA, from combinatorial technologies at the level of encoding DNA 15 sequence (such as DNA shuffling, phage display/selection), or from computer-aided design studies, followed by the validation for the desired activities as described in the prior art.

Specific, non-conservative mutations can be also introduced in the polypeptides 20 of the invention with different purposes. Mutations reducing the affinity of the chemokine-like polypeptide for a receptor may increase its ability to be reused and recycled, potentially increasing its therapeutic potency (Robinson CR, 2002). Immunogenic epitopes eventually present in the polypeptides of the invention can be exploited for developing vaccines (Stevanovic S, 2002), or eliminated by modifying their sequence following known methods for selecting mutations for increasing protein

stability, and correcting them (van den Burg B and Eijsink V, 2002; WO 02/05146, WO 00/34317, WO 98/52976).

Further alternative polypeptides of the invention are active fragments, precursors, salt, or derivative of the amino acid sequences the above described sequences .

5 Fragments should present deletions of terminal or internal amino acids not altering their function, and should involve generally a few amino acids, e.g., under ten, and preferably under three, without removing or displacing amino acids which are critical to the functional conformation of the proteins.

10 The "precursors" are compounds which can be converted into the compounds of present invention by metabolic and enzymatic processing prior or after the administration to the cells or to the body.

15 The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the polypeptides of the present invention. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids such as, for example, acetic acid or oxalic acid. Any 20 of such salts should have substantially similar activity to the peptides and polypeptides of the invention or their analogs.

25 The term "derivatives" as herein used refers to derivatives which can be prepared from the functional groups present on the lateral chains of the amino acid moieties or on the amino- or carboxy-terminal groups according to known methods. Such molecules can result also from other modifications which do not normally alter primary

- 12 -

sequence, for example *in vivo* or *in vitro* chemical derivatization of polypeptides (acetylation or carboxylation), those made by modifying the pattern of phosphorylation

(introduction of phosphotyrosine, phosphoserine, or phosphothreonine residues) or glycosylation (by exposing the polypeptide to mammalian glycosylating enzymes) of a

5 peptide during its synthesis and processing or in further processing steps. Alternatively, derivatives may include esters or aliphatic amides of the carboxyl-groups and N-acyl derivatives of free amino groups or O-acyl derivatives of free hydroxyl-groups and are formed with acyl-groups as for example alcanoyle- or aryl-groups.

The generation of the derivatives may involve a site-directed modification of an

10 appropriate residue, in an internal or terminal position. The residues used for attachment should they have a side-chain amenable for polymer attachment (i.e., the side chain of an amino acid bearing a functional group, e.g. lysine, aspartic acid, glutamic acid, cysteine, histidine, etc.). Alternatively, a residue having a side chain amenable for polymer attachment can replace an amino acid of the polypeptide, or can

15 be added in an internal or terminal position of the polypeptide. Also, the side chains of the genetically encoded amino acids can be chemically modified for polymer attachment, or unnatural amino acids with appropriate side chain functional groups can be employed. The preferred method of attachment employs a combination of peptide synthesis and chemical ligation. Advantageously, the attachment of a water-soluble

20 polymer will be through a biodegradable linker, especially at the amino-terminal region of a protein. Such modification acts to provide the protein in a precursor (or "pro-drug") form, that, upon degradation of the linker releases the protein without polymer modification.

Polymer attachment may be not only to the side chain of the amino acid naturally

25 occurring in a specific position of the antagonist or to the side chain of a natural or

- 13 -

unnatural amino acid that replaces the amino acid naturally occurring in a specific position of the antagonist, but also to a carbohydrate or other moiety that is attached to the side chain of the amino acid at the target position. Rare or unnatural amino acids can be also introduced by expressing the protein in specifically engineered bacterial 5 strains (Bock A, 2001).

Variants of the polypeptides above indicated can be naturally occurring, being identified in organisms other than humans, or resulting from the translation of a single nucleotide polymorphism. Alternatively, artificial variants can be prepared by chemical synthesis, by site-directed mutagenesis techniques, or any other known technique 10 suitable thereof, which provide a finite set of substantially corresponding mutated or shortened peptides or polypeptides which can be routinely obtained and tested by one of ordinary skill in the art using the teachings presented in the prior art.

The novel amino acid sequences disclosed in the present patent application can be used to provide different kind of reagents and molecules. Examples of these 15 compounds are binding proteins or antibodies that can be identified using their full sequence or specific fragments, such as antigenic determinants. Peptide libraries can be used in known methods (Tribbick G, 2002) for screening and characterizing antibodies or other proteins binding the claimed amino acid sequences, and for identifying alternative forms of such polypeptides having similar binding properties.

20 The present patent application discloses also fusion proteins comprising any of the polypeptides described above. These polypeptides should contain protein sequence heterologous to the one disclosed in the present patent application, without significantly impairing the chemotactic activity and possibly providing additional properties. Examples of such properties are an easier purification procedure, a longer 25 lasting half-life in body fluids, an additional binding moiety, the maturation by means of

an endoproteolytic digestion, or extracellular localization. This latter feature is of particular importance for defining a specific group of fusion or chimeric proteins included in the above definition since it allows the claimed molecules to be localized in the space where not only isolation and purification of these polypeptides is facilitated, 5 but also where generally chemokines and their receptor interact.

Design of the moieties, ligands, and linkers, as well methods and strategies for the construction, purification, detection and use of fusion proteins are disclosed in the literature (Nilsson J et al., 1997; Methods Enzymol, Vol. 326-328, Academic Press, 2000). The preferred protein sequences that can be comprised in the fusion proteins of 10 the invention belong to these protein sequences: membrane-bound protein, immunoglobulin constant region, multimerization domains, extracellular proteins, signal peptide-containing proteins, export signal-containing proteins. Features of these sequences and their specific uses are disclosed in a detailed manner, for example, for albumin fusion proteins (WO 01/77137), fusion proteins including multimerization 15 domain (WO 01/02440, WO 00/24782, WO 94/10308, WO 97/30161), immuno-conjugates (Garnett MC, 2001), or fusion protein including sequences allowing the purification of the recombinant products by affinity chromatography (Constans A, 2002; Burgess RR and Thompson NE, 2002; Lowe CR et al., 2001; Sheibani N, 1999).

Several studies on structure-activity features of chemokines indicate that these 20 proteins bind and activate receptors by making use of the amino-terminal region. Proteolytic digestion, mutagenesis, or chemical modifications directed to amino acids in this region can generate compounds having antagonistic activity (Loetscher P and Clark-Lewis I, 2001; Lambeir A et al., 2001, Proost P et al., 2001). Thus, antagonistic molecules resulting from specific modifications (deletions, non-conservative 25 substitutions, addition of chemical groups) of one or more residues in the amino-

- 15 -

terminal region or in other regions of the corresponding chemokine are considered having therapeutic potential for inflammatory and autoimmune diseases (WO 02/28419; WO 00/27880; WO 99/33989; Schwarz MK and Wells T, 1999). Therefore, a further object of the present patent application is represented by such kind of 5 antagonists generated by modifying the polypeptides of the invention.

The polypeptides of the invention can be used to generate and characterize ligands binding specifically to them. These molecules can be natural or artificial, very different from the chemical point of view (binding proteins, antibodies, molecularly imprinted polymers), and can be produced by applying the teachings in the art (WO 10 02/74938; Kuroiwa Y et al., 2002; Haupt K, 2002; van Dijk MA and van de Winkel JG, 2001; Gavilondo JV and Larrick JW, 2000). Such ligands can antagonize or inhibit the chemotactic activity of the polypeptide against which they have been generated. In particular, common and efficient ligands are represented by extracellular domain of a membrane-bound protein or antibodies, which can be in the form monoclonal, 15 polyclonal, humanized antibody, or an antigen-binding fragment.

The polypeptides and the polypeptide-based derived reagents described above can be in alternative forms, according to the desired method of use and/or production, such as active conjugates or complexes with a molecule chosen amongst radioactive labels, fluorescent labels, biotin, or cytotoxic agents.

20 Specific molecules, such as peptide mimetics, can be also designed on the sequence and/or the structure of a polypeptide of the invention. Peptide mimetics (also called peptidomimetics) are peptides chemically modified at the level of amino acid side chains, of amino acid chirality, and/or of the peptide backbone. These alterations are intended to provide agonists or antagonists of the polypeptides of the invention with 25 improved preparation, potency and/or pharmacokinetics features.

For example, when the peptide is susceptible to cleavage by peptidases following injection into the subject is a problem, replacement of a particularly sensitive peptide bond with a non-cleavable peptide mimetic can provide a peptide more stable and thus more useful as a therapeutic. Similarly, the replacement of an L-amino acid residue is a 5 standard way of rendering the peptide less sensitive to proteolysis, and finally more similar to organic compounds other than peptides. Also useful are amino-terminal blocking groups such as t-butyloxycarbonyl, acetyl, theyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4-dinitrophenyl. Many other 10 modifications providing increased potency, prolonged activity, easiness of purification, and/or increased half-life are disclosed in the prior art (WO 02/10195; Villain M et al., 2001).

Preferred alternative, synonymous groups for amino acids derivatives included in peptide mimetics are those defined in Table II. A non-exhaustive list of amino acid 15 derivatives also include aminoisobutyric acid (Aib), hydroxyproline (Hyp), 1,2,3,4-tetrahydro-isoquinoline-3-COOH, indoline-2carboxylic acid, 4-difluoro-proline, L-thiazolidine-4-carboxylic acid, L-homoproline, 3,4-dehydro-proline, 3,4-dihydroxy-phenylalanine, cyclohexyl-glycine, and phenylglycine.

By "amino acid derivative" is intended an amino acid or amino acid-like chemical 20 entity other than one of the 20 genetically encoded naturally occurring amino acids. In particular, the amino acid derivative may contain substituted or non-substituted, linear, branched, or cyclic alkyl moieties, and may include one or more heteroatoms. The amino acid derivatives can be made *de novo* or obtained from commercial sources (Calbiochem-Novabiochem AG, Switzerland; Bachem, USA).

Various methodologies for incorporating unnatural amino acids derivatives into proteins, using both *in vitro* and *in vivo* translation systems, to probe and/or improve protein structure and function are disclosed in the literature (Dougherty DA, 2000). Techniques for the synthesis and the development of peptide mimetics, as well as non - 5 peptide mimetics, are also well known in the art (Golebiowski A et al., 2001; Hruby VJ and Balse PM, 2000; Sawyer TK, in "Structure Based Drug Design", edited by Veerapandian P, Marcel Dekker Inc., pg. 557-663, 1997).

Another object of the present invention are isolated nucleic acids encoding for the polypeptides of the invention having chemotactic activity, the polypeptides binding to 10 an antibody or a binding protein generated against them, the corresponding fusion proteins, or mutants having antagonistic activity as disclosed above. Preferably, these nucleic acids should comprise a DNA sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 15, or the complement of said DNA sequences.

Alternatively, the nucleic acids of the invention should hybridize under high 15 stringency conditions, or exhibits at least about 85% identity over a stretch of at least about 30 nucleotides, with a nucleic acid selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 15 or a complement of said DNA sequences. A further object of the present invention is therefore the polypeptides encoded by these purified nucleic acids.

20 The wording "high stringency conditions" refers to conditions in a hybridization reaction that facilitate the association of very similar molecules and consist in the overnight incubation at 60°-65°C in a solution comprising 50 % formamide, 5X SSC (150 m M NaCl, 15 m M trisodium citrate), 50 mM sodium phosphate (p H 7 6), 5x Denhardt's solution, 10 % dextran sulphate, and 20 microgram/ml denatured, sheared

salmon sperm DNA, followed by washing the filters in 0.1X SSC at the same temperature.

These nucleic acids, including nucleotide sequences substantially the same, can be comprised in plasmids, vectors and any other DNA construct which can be used for 5 maintaining, modifying, introducing, or expressing the encoded polypeptide. In particular, vectors wherein said nucleic acid molecule is operatively linked to expression control sequences can allow expression in prokaryotic or eukaryotic host cells of the encoded polypeptide.

The wording "nucleotide sequences substantially the same" includes all other 10 nucleic acid sequences that, by virtue of the degeneracy of the genetic code, also code for the given amino acid sequences. In this sense, the literature provides indications on preferred or optimized codons for recombinant expression (Kane JF et al., 1995).

The nucleic acids and the vectors can be introduced into cells with different purposes, generating transgenic cells and organisms. A process for producing cells 15 capable of expressing a polypeptide of the invention comprises genetically engineering cells with such vectors and nucleic acids.

In particular, host cells (e.g. bacterial cells) can be modified by transformation for allowing the transient or stable expression of the polypeptides encoded by the nucleic acids and the vectors of the invention. Alternatively, said molecules can be used to 20 generate transgenic animal cells or non-human animals (by non- / homologous recombination or by any other method allowing their stable integration and maintenance), having a constitutive or inducible altered expression levels (i.e. enhanced or reduced) of the polypeptides of the invention, when the level is compared with the normal expression levels. Such precise modifications can be obtained by 25 making use of the nucleic acids of the inventions and of technologies associated, for

example, to gene therapy (Meth. Enzymol., vol. 346, 2002) or to site-specific recombinases (Kolb AF, 2002). Model systems based on the chemokine-like polypeptides disclosed in the present patent application for the systematic study of their function can be also generated by gene targeting into human cell lines (Bunz F, 2002).

5 The polypeptides of the invention can be prepared by any method known in the art, including recombinant DNA-related technologies, and chemical synthesis technologies. In particular, a method for making a polypeptide of the invention may comprise culturing a host or transgenic cell as described above under conditions in which the nucleic acid or vector is expressed, and recovering the polypeptide encoded
10 by said nucleic acid or vector from the culture. For example, when the vector expresses the polypeptide as a fusion protein with an extracellular or signal-peptide containing proteins, the recombinant product can be secreted in the extracellular space, and can be more easily collected and purified from cultured cells in view of further processing or, alternatively, the cells can be directly used or administered.

15 The DNA sequence coding for the proteins of the invention can be inserted and ligated into a suitable episomal or non- / homologously integrating vectors, which can be introduced in the appropriate host cells by any suitable means (transformation, transfection, conjugation, protoplast fusion, electroporation, calcium phosphate-precipitation, direct microinjection, etc.). Factors of importance in selecting a particular
20 plasmid or viral vector include: the ease with which recipient cells that contain the vector, may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

- 20 -

The vectors should allow the expression of the isolated or fusion protein including the polypeptide of the invention by prokaryotic or eukaryotic host cells under the control of transcriptional initiation / termination regulatory sequences, which are chosen to be constitutively active or inducible in said cell. A cell line substantially enriched in such cells can be then isolated to provide a stable cell line.

Different transcriptional and translational regulatory sequences may be employed for eukaryotic hosts (e.g. yeasts, insect, plant, or mammalian cells), depending on the nature of the host. They may be derived from viral sources, such as adenovirus, bovine papilloma virus, Simian virus or the like, where the regulatory signals are associated with a particular gene which has a high level of expression. Examples are the TK promoter of the Herpes virus, the SV40 early promoter, the yeast GAL4 gene promoter, etc. Transcriptional initiation regulatory signals may be selected which allow for repression and activation, so that expression of the genes can be modulated. The cells stably transformed by the introduced DNA can be selected by introducing one or more markers allowing the selection of host cells that contain the expression vector. The marker may also provide for phototrophy to an auxotrophic host, biocide resistance, e.g. antibiotics, or heavy metals such as copper, or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection.

Host cells may be either prokaryotic or eukaryotic. Preferred are eukaryotic hosts, e.g. mammalian cells, such as human, monkey, mouse, and Chinese Hamster Ovary (CHO) cells, because they provide post-translational modifications to proteins, including correct folding and glycosylation. Also yeast cells can carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of

plasmids that can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences in cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides).

The above mentioned embodiments of the invention can be achieved by
5 combining the disclosure provided by the present patent application on the sequence of
novel chemokine-like polypeptides with the knowledge of common molecular biology
techniques.

Many books and reviews provides teachings on how to clone and produce
recombinant proteins using vectors and prokaryotic or eukaryotic host cells, such as
10 some titles in the series "A Practical Approach" published by Oxford University Press
("DNA Cloning 2: Expression Systems", 1995; "DNA Cloning 4: Mammalian Systems",
1996; "Protein Expression", 1999; "Protein Purification Techniques", 2001).

Moreover, updated and more focused literature provides an overview of the
technologies for expressing polypeptides in a high-throughput manner (Chambers SP,
15 2002; Coleman TA, et al., 1997), of the cell systems and the processes used
industrially for the large-scale production of recombinant proteins having therapeutic
applications (Andersen DC and Krummen L, 2002; Chu L and Robinson DK, 2001),
and of alternative eukaryotic expression systems for expressing the polypeptide of
interest, which may have considerable potential for the economic production of the
20 desired protein, such the ones based on transgenic plants (Giddings G, 2001) or the
yeast *Pichia pastoris* (Lin Cereghino GP et al., 2002). Recombinant protein products
can be rapidly monitored with various analytical technologies during purification to
verify the amount and the quantity of the expressed polypeptides (Baker KN et al.,
2002), as well as to check if there is problem of bioequivalence and immunogenicity
25 (Schellekens H, 2002; Gendel SM, 2002).

Totally synthetic chemokines are disclosed in the literature (Brown A et al., 1996), and many examples of chemical synthesis technologies, which can be effectively applied for the chemokine-like polypeptides of the invention given their short length, are available in the literature, as solid phase or liquid phase synthesis technologies. for 5 example, the amino acid corresponding to the carboxy-terminus of the peptide to be synthetized is bound to a support which is insoluble in organic solvents, and by alternate repetition of reactions, one wherein amino acids with their amino groups and side chain functional groups protected with appropriate protective groups are condensed one by one in order from the carboxy-terminus to the amino-terminus, and 10 one where the amino acids bound to the resin or the protective group of the amino groups of the peptides are released, the peptide chain is thus extended in this manner.

Solid phase synthesis methods are largely classified by the tBoc method and the Fmoc method, depending on the type of protective group used. Typically used protective groups include tBoc (t-butoxycarbonyl), Cl-Z (2-chlorobenzylloxycarbonyl), 15 Br-Z (2-bromobenzylloxycarbonyl), Bzl (benzyl), Fmoc (9-fluorenylmethoxycarbonyl), Mbh (4,4'-dimethoxydibenzhydryl), Mtr (4-methoxy-2,3,6-trimethylbenzenesulphonyl), Trt (trityl), Tos (tosyl), Z (benzyloxycarbonyl) and Cl2-Bzl (2,6-dichlorobenzyl) for the amino groups; NO2 (nitro) and Pmc (2,2,5,7,8-pentamethylchromane-6-sulphonyl) for the guanidino groups; and tBu (t-butyl) for the hydroxyl groups). After synthesis of the 20 desired peptide, it is subjected to the de-protection reaction and cut out from the solid support. Such peptide cutting reaction may be carried with hydrogen fluoride or trifluoromethane sulfonic acid for the Boc method, and with TFA for the Fmoc method.

The purification of the polypeptides of the invention can be carried out by any one of the methods known for this purpose, i.e. any conventional procedure involving 25 extraction, precipitation, chromatography, electrophoresis, or the like. A further

purification procedure that may be used in preference for purifying the protein of the invention is affinity chromatography using monoclonal antibodies or affinity groups, which bind the target protein and which are produced and immobilized on a gel matrix contained within a column. Impure preparations containing the proteins are passed through the column. The protein will be bound to the column by heparin or by the specific antibody while the impurities will pass through. After washing, the protein is eluted from the gel by a change in pH or ionic strength. Alternatively, HPLC (High Performance Liquid Chromatography) can be used. The elution can be carried using a water-acetonitrile-based solvent commonly employed for protein purification.

10 The disclosure of the novel polypeptides of the invention, and the reagents disclosed in connection to them (antibodies, nucleic acids, cells) allows also to screen and characterize compounds that enhance or reduce their expression level into a cell or in an animal. Examples of compounds that can reduce or block the expression of the chemokine-like polypeptides are antisense oligonucleotides (Stein CA, 2001) or small
15 interfering, double stranded RNA molecules that can trigger RNA interference-mediated silencing (Paddison PJ et al., 2002; Lewis DL et al., 2002). These compounds are intended as antagonists (in addition to the ones above described in connection to mutants and ligands) in the context of the possible mechanism of antagonism for blocking cytokine/chemokine-controlled pathways as defined in the
20 literature (Choy EH and Panayi GS, 2001; Dower SK, 2000).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands that may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A
25 synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

The invention includes purified preparations of the compounds of the invention (polypeptides, nucleic acids, cells, etc.). Purified preparations, as used herein, refers to the preparations containing at least 1%, preferably at least 5%, by dry weight of the compound of the invention.

5 The present patent application discloses a series of novel chemokine-like polypeptides and of related reagents having several possible applications. In particular, whenever an increase in the chemotactic activity of a polypeptide of the invention is desirable in the therapy or in the prevention of a disease, reagents such as the disclosed chemokine-like polypeptides, the corresponding fusion proteins and peptide
10 mimetics, the encoding nucleic acids, the expressing cells, or the compounds enhancing their expression can be used.

Therefore, the present invention discloses pharmaceutical compositions for the treatment or prevention of diseases needing an increase in the chemotactic activity of a polypeptide of the invention, which contain one of the disclosed chemokine-like
15 polypeptides, the corresponding fusion proteins and peptide mimetics, the encoding nucleic acids, the expressing cells, or the compounds enhancing their expression, as active ingredient.

The process for the preparation of these pharmaceutical compositions comprises combining the disclosed chemokine-like polypeptides, the corresponding fusion
20 proteins and peptide mimetics, the encoding nucleic acids, the expressing cells, or the compounds enhancing their expression, together with a pharmaceutically acceptable carrier.

Methods for the treatment or prevention of diseases needing an increase in the chemotactic activity of a polypeptide of the invention, comprise the administration of a
25 therapeutically effective amount of the disclosed chemokine-like polypeptides, the

corresponding fusion proteins and peptide mimetics, the encoding nucleic acids, the expressing cells, or the compounds enhancing their expression.

Amongst the reagents disclosed in the present patent application, the ligands, the antagonists or the compounds reducing the expression or the activity of polypeptides of 5 the invention have several applications, and in particular they can be used in the therapy or in the diagnosis of a disease associated to the excessive chemotactic activity of a polypeptide of the invention.

Therefore, the present invention discloses pharmaceutical compositions for the treatment or prevention of diseases associated to the excessive chemotactic activity of 10 a polypeptide of the invention, which contain one of the ligands, antagonists, or compounds reducing the expression or the activity of such polyptides, as active ingredient.

The process for the preparation of these pharmaceutical compositions comprises combining the ligand, the antagonist, or the compound, together with a 15 pharmaceutically acceptable carrier.

Methods for the treatment or prevention of diseases associated to the excessive chemotactic activity of the polypeptide of the invention comprise the administration of a therapeutically effective amount of the antagonist, the ligand or of the compound.

The pharmaceutical compositions of the invention may contain, in addition to 20 chemokine-like polypeptide or to the related reagent, suitable pharmaceutically acceptable carriers, biologically compatible vehicles and additives which are suitable for administration to an animal (for example, physiological saline) and eventually comprising auxiliaries (like excipients, stabilizers, adjuvants, or diluents) which facilitate the processing of the active compound into preparations which can be used 25 pharmaceutically.

The pharmaceutical compositions may be formulated in any acceptable way to meet the needs of the mode of administration. For example, of biomaterials, sugar-macromolecule conjugates, hydrogels, polyethylene glycol and other natural or synthetic polymers can be used for improving the active ingredients in terms of drug 5 delivery efficacy. Technologies and models to validate a specific mode of administration are disclosed in literature (Davis BG and Robinson MA, 2002; Gupta P et al., 2002; Luo B and Prestwich GD, 2001; Cleland JL et al., 2001; Pillai O and Panchagnula R, 2001).

Polymers suitable for these purposes are biocompatible, namely, they are non- 10 toxic to biological systems, and many such polymers are known. Such polymers may be hydrophobic or hydrophilic in nature, biodegradable, non-biodegradable, or a combination thereof. These polymers include natural polymers (such as collagen, gelatin, cellulose, hyaluronic acid), as well as synthetic polymers (such as polyesters, polyorthoesters, polyanhydrides). Examples of hydrophobic non-degradable polymers 15 include polydimethyl siloxanes, polyurethanes, polytetrafluoroethylenes, polyethylenes, polyvinyl chlorides, and polymethyl methacrylates. Examples of hydrophilic non-degradable polymers include poly(2-hydroxyethyl methacrylate), polyvinyl alcohol, poly(N-vinyl pyrrolidone), polyalkylenes, polyacrylamide, and copolymers thereof. Preferred polymers comprise as a sequential repeat unit ethylene oxide, such as 20 polyethylene glycol (PEG).

Any accepted mode of administration can be used and determined by those skilled in the art to establish the desired blood levels of the active ingredients. For example, administration may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, oral, or 25 buccal routes. The pharmaceutical compositions of the present invention can also be

administered in sustained or controlled release dosage forms, including depot injections, osmotic pumps, and the like, for the prolonged administration of the polypeptide at a predetermined rate, preferably in unit dosage forms suitable for single administration of precise dosages.

5 Parenteral administration can be by bolus injection or by gradual perfusion over time. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients known in the art, and can be prepared according to routine methods. In addition, suspension of the active compounds as appropriate oily injection suspensions
10 may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions that may contain substances increasing the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran.
15 Optionally, the suspension may also contain stabilizers. Pharmaceutical compositions include suitable solutions for administration by injection, and contain from about 0.01 to 99.99 percent, preferably from about 20 to 75 percent of active compound together with the excipient.

The wording "therapeutically effective amount" refers to an amount of the active
20 ingredients that is sufficient to affect the course and the severity of the disease, leading to the reduction or remission of such pathology. The effective amount will depend on the route of administration and the condition of the patient.

The wording "pharmaceutically acceptable" is meant to encompass any carrier,
25 which does not interfere with the effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which is administered. For example, for

parenteral administration, the above active ingredients may be formulated in unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution. Carriers can be selected also from starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium 5 stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the various oils, including those of petroleum, animal, vegetable or synthetic origin (peanut oil, soybean oil, mineral oil, sesame oil).

It is understood that the dosage administered will be dependent upon the age, 10 sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art. The total dose required for each treatment may be administered by multiple doses or in a single dose. The pharmaceutical composition of the present invention may be administered 15 alone or in conjunction with other therapeutics directed to the condition, or directed to other symptoms of the condition. Usually a daily dosage of active ingredient is comprised between 0.01 to 100 milligrams per kilogram of body weight per day. Ordinarily 1 to 40 milligrams per kilogram per day given in divided doses or in sustained release form is effective to obtain the desired results. Second or subsequent 20 administrations can be performed at a dosage, which is the same, less than, or greater than the initial or previous dose administered to the individual.

Apart from the methods having a therapeutic or a production purpose, several other methods can make use of the chemokine-like polypeptides and of the related reagents disclosed in the present patent application.

In a first example, a method for screening candidate compounds effective to treat a disease related to a chemokine-like polypeptides of the invention, comprises:

- (a) contacting host cells expressing such polypeptide, transgenic non-human animals, or transgenic animal cells having enhanced or reduced expression levels of the polypeptide, with a candidate compound; and
- (b) determining the effect of the compound on the animal or on the cell.

In a second example, a method for identifying a candidate compound as an antagonist/inhibitor or agonist/activator of a polypeptide of the invention comprises:

- (a) contacting the polypeptide, the compound, and a mammalian cell or a mammalian cell membrane; and
- (b) measuring whether the molecule blocks or enhances the interaction of the polypeptide, or the response that results from such interaction, with the mammalian cell or the mammalian cell membrane.

In a third example, methods for determining the activity and/or the presence of the polypeptide of the invention in a sample, can detect either the polypeptide or the encoding RNA/DNA. Thus, such a method comprises:

- (a) providing a protein-containing sample;
- (b) contacting said sample with a ligand of the invention; and
- (c) determining the presence of said ligand bound to said polypeptide, thereby determining the activity and/or the presence of polypeptide in said sample.

Alternatively, the method comprises:

- (a) providing a nucleic acids-containing sample;
- (b) contacting said sample with a nucleic acid of the invention; and
- (c) determining the hybridization of said nucleic acid with a nucleic acid into the sample, thereby determining the presence of the nucleic acid in the sample.

- 30 -

In this context, primer sequences containing the sequences SEQ ID NO: 17-28 (Table III) can be used as well for determining the presence or the amount of a transcript or of a nucleic acid encoding a polypeptide of invention in a sample by means of Polymerase Chain Reaction amplification.

5 A further object of the present invention are kits for measuring the activity and/or the presence of chemokine-like polypeptide of the invention in a sample comprising one or more of the reagents disclosed in the present patent application: a chemokine-like polypeptide of the invention, an antagonist, ligand or peptide mimetic, an isolated nucleic acid or the vector, a pharmaceutical composition, an expressing cell, a 10 compound increasing or decreasing the expression levels, and/or primer sequences containing the sequences SEQ ID NO: 17-28.

Those kits can be used for *in vitro* diagnostic or screenings methods, and their actual composition should be adapted to the specific format of the sample (e.g. biological sample tissue from a patient), and the molecular species to be measured.

15 For example, if it is desired to measure the concentration of the chemokine-like polypeptide, the kit may contain an antibody and the corresponding protein in a purified form to compare the signal obtained in Western blot. Alternatively, if it is desired to measure the concentration of the transcript for the chemokine-like polypeptide, the kit may contain a specific nucleic acid probe designed on the corresponding ORF 20 sequence, or may be in the form of nucleic acid array containing such probe, or the primer sequences disclosed as SEQ ID NO: 17-28 (Table III). The kits can be also in the form of protein-, peptide mimetic-, or cell-based microarrays (Templin MF et al., 2002; Pellois JP et al., 2002; Blagoev B and Pandey A, 2001); allowing high-throughput proteomics studies, by making use of the proteins, peptide mimetics and cells 25 disclosed in the present patent application.

The novel chemokine-like polypeptides of the invention and the related reagents that may be useful, as active ingredients in pharmaceutical compositions appropriately formulated, in the treatment or prevention of diseases such as cell proliferative disorders, autoimmune/inflammatory disorders, cardiovascular disorders, neurological disorders, developmental disorders, metabolic disorder, infections and other pathological conditions. In particular, given the known properties of chemokines, the disclosed polypeptides and reagents should address conditions involving abnormal or defective cell migration. Non-limitative examples of such conditions are the following: 5 arthritis, rheumatoid arthritis (RA), psoriatic arthritis, osteoarthritis, systemic lupus erythematosus (SLE), systemic sclerosis, scleroderma, polymyositis, 10 glomerulonephritis, fibrosis, lung fibrosis and inflammation, allergic or hypersensitivity diseases, dermatitis, asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), Crohn's diseases, ulcerative colitis, multiple sclerosis, septic shock, HIV infection, transplant rejection, wound healing, metastasis, 15 endometriosis, hepatitis, liver fibrosis, cancer, analgesia, and vascular inflammation related to atherosclerosis.

The therapeutic applications of the polypeptides of the invention and of the related reagents can be evaluated (in terms of safety, pharmacokinetics and efficacy) by the means of the *in vivo* or *in vitro* assays making use of animal cell, tissues and 20 models (Coleman RA et al., 2001; Li AP, 2001; Methods Mol. Biol vol. 138, "Chemokines Protocols", edited by Proudfoot A et al., Humana Press Inc., 2000; Methods Enzymol, vol. 287 and 288, Academic Press, 1997), or by the means of *in silico*, computational approaches (Johnson DE and Wolfgang GH, 2000), known for the validation of chemokines and other biological products during drug discovery and 25 preclinical development.

- 32 -

All publications, patents and patent applications cited herein are incorporated in full by reference for all purposes.

The invention will now be described with reference to the specific embodiments by means of the following Examples, which should not be construed as in any way

5 limiting the present invention. The content of the description comprises all modifications and substitutions which can be practiced by a person skilled in the art in light of the above teachings and, therefore, without extending beyond the meaning and purpose of the claims.

10

EXAMPLES

Example 1: Selection of chemokine-like open reading frames (ORFs) from human genome

Perl (Practical Extraction and Report Language) is a programming language
15 having powerful pattern matching functions into large text data files allowing the extraction of information from genomic DNA sequences, starting from an alpha-numerical expression describing a defined consensus sequence (Stein LD, 2001).

A Perl script was used to retrieve novel open reading frames (ORFs), having chemokine-like features, in a FASTA-formatted sequence file containing the NCBI
20 genome (build 28). After translating the genomic DNA sequence into the six possible reading frames (3 forward and 3 reverse), each of these translated sequences was then tested for a match against a pattern designed to detect to chemokine -like proteins, which was elaborated comparing multiple sequence alignments of known chemokines.

The following pattern, fitting all the aligned sequences, was adopted:

25

- 33 -

{M}-{X}₃₋₁₂ -{L or I or V}₁₋₃ -{X}₀₋₂ -{L or I or V}₂₋₄ -{X}₀₋₂ -{L or I or V}₁₋₃ -{X}₁₀₋₃₀ -{C}-(X)₀₋₃ -C- (X)₂₀₋₄₀ - C -{X}₁₂₋₂₀ -{C}-{X}₁₅₋₄₀ STOP

The letter(s) between brackets represented alternative amino acids (in one-letter code) which should be present the number of times indicated in subscript characters. This expression, which describes the entire family of sequences on the basis of the respective positioning of the initial methionine, hydrophobic residues, and conserved cysteines on the linear sequence, can be transformed in Perl language as follows:

10 M[^*]{3,12}[LIV]{1,3}[^*]{0,2}[LIV]{2,4}[^*]{0,2}[LIV]{1,3}[^*]{10,30}C[^*]{0,3}C[^*]{20,40}C[^*]{12,20}C[^*]{15,40}[*]

A total of FASTA-formatted 7974 ORFs matching the pattern were compared to known proteins present in protein databases (SwissProt/Trembl and Derwent 15 GENESEQ) using a rapid searching program for local alignments between a query and a hit sequence based on Basic Local Alignment Search Tool (BLAST, BLASTX) and ClustalW algorithms (Altschul SF et al., 1990; Pearson WR and Miller W, 1992; Gish W and States DJ, 1993). BLAST parameters used were: Comparison matrix = BLOSUM62; word length = 3; .E value cutoff = 10; Gap opening and extension = 20 default; No filter.

The sequences obtained from this first screening were further selected using additional criteria. 2441 ORFs showing at least 70% of homology with known proteins in protein databases were eliminated. The remaining 5533 ORFs were filtered using 25 neural network-based algorithms developed for the prediction (probability at least 0.7) of a N-terminal signal peptide and of an alpha helix secondary structure having at least

5 amino acids within the C-terminal 30 amino acids (a hallmark of the IL8-like fold) with high confidence. The resulting 253 ORFs, which were predicted as containing these features, were transformed in text format and were compared to known chemokines, searching manually for the best alignments. This further refinement, based on the 5 qualitative assessment of the alignments, led to the selection of eight chemokine-like encoding ORFs for which all criteria for the prediction (sequence length, cysteine spacing, N-terminal signal sequence, C-terminal alpha helix) were fulfilled, making them comparable to known chemokines.

The DNA sequence GNSQ_1754 (SEQ ID NO: 1), belonging to human 10 chromosome 13, contains an ORF encoding for the 98-amino acid long protein sequence p1754 (SEQ ID NO: 2), which, according to the prediction, presents a 22-amino acid long signal sequence and an alpha helix covering the residues 70-79 (figure 1).

The DNA sequence GNSQ_0711 (SEQ ID NO: 3), belonging to human 15 chromosome 16, contains an ORF encoding for the 109 -amino acid long protein sequence p0711 (SEQ ID NO: 4), which, according to the prediction, presents a 17-amino acid long signal sequence and an alpha helix covering the residues 98-106 (figure 2).

The DNA sequence GNSQ_2882 (SEQ ID NO: 5), belonging to human 20 chromosome 6, contains an ORF encoding for the 107 -amino acid long protein sequence p2882 (SEQ ID NO: 6), which, according to the prediction, presents a 18 -amino acid long signal sequence and an alpha helix covering the residues 96-104 (figure 3).

The DNA sequence GNSQ_4711 (SEQ ID NO: 7), belonging to human 25 chromosome 3, contains an ORF encoding for the 102 -amino acid long protein

sequence p4711 (SEQ ID NO: 8), which, according to the prediction, presents a 22-amino acid long signal sequence and an alpha helix covering the residues 83-97 (figure 4).

The DNA sequence GNSQ_4320 (SEQ ID NO: 9), belonging to human 5 chromosome 3, contains an ORF encoding for the 101-amino acid long protein sequence p4320 (SEQ ID NO: 10), which, according to the prediction, presents a 16-amino acid long signal sequence and an alpha helix covering the residues 90-98 (figure 5).

The DNA sequence GNSQ_5008 (SEQ ID NO: 11), belonging to human 10 chromosome 12, contains an ORF encoding for the 112-amino acid long protein sequence p5008 (SEQ ID NO: 12), which, according to the prediction, presents a 17-amino acid long signal sequence and an alpha helix covering the residues 95-109 (figure 6).

The DNA sequence GNSQ_0210 (SEQ ID NO: 13), belonging to human 15 chromosome 7, contains an ORF encoding for the 127-amino acid long protein sequence p0210 (SEQ ID NO: 14), which, according to the prediction, presents a 16-amino acid long signal sequence and an alpha helix covering the residues 94-113 (figure 7).

The DNA sequence GNSQ_4922 (SEQ ID NO: 15), belonging to human 20 chromosome 10, contains an ORF encoding for the 91-amino acid long protein sequence p4922 (SEQ ID NO: 14), which, according to the prediction, presents a 23-amino acid long signal sequence and an alpha helix covering the residues 67-74 (figure 8).

Amongst these sequences characterized as encoding chemokine-like 25 polypeptides, five of them (p1754, p0711, p2882, p0210, and p4922) present a central

Cys-rich region in which the first two Cysteines are separated by 1-3 amino acids, and can be compared with known C-X-C chemokines (figure 9). The remaining three sequences (p4711, p4320, and p5008) present two adjacent Cysteines at the beginning of such region, and therefore can be compared with known C-C chemokines
5 (figure 10).

Example 2: Cloning of the novel chemokine-like ORFs from human genomic DNA

Six of the eight above-defined chemokine-like ORFs (GNSQ_1754, GNSQ_4922, GNSQ_5008, GNSQ_0210, GNSQ_0711, and GNSQ_4320) were first cloned from
10 human genomic DNA into a cloning vector, and then transferred into an expression vector using Polymerase Chain Reaction (PCR), with pairs of forward / reverse primers specific for each ORF (see arrows in figure 1, 2, and 5-8).

The cloning primers (CL series; Table III), having a length comprised between 19 and 25 bases, were designed for amplifying each ORF, using human genomic DNA as
15 template. The forward primers start from the initial ATG or a few nucleotides before. The reverse primers are complementary to the 3' end of the ORF, including the stop codon.

The PCR was performed by mixing the following components in each ORF-specific reaction (total volume of 50 μ l in double-distilled water):

20 150 ng human genomic DNA (Clontech)
1.2 μ M primers (0.6 μ M each primer)
240 μ M dNTP (Invitrogen)
0.5 μ l AmpliTaq (2.5 Units; Applied Biosystems)
5 μ l AmpliTaq buffer 10X (Applied Biosystems)

The PCR reactions were performed using an initial denaturing step if 94 °C for 2 minutes, followed by 30 cycles:

94°C for 30 seconds

55°C for 30 seconds

5 72°C for 30 seconds

After a final elongation step of 72°C for 10 minutes, the PCR products were directly subcloned into the pCRII-TOPO vector using the TOPO™ cloning system (Invitrogen), according to manufacturer's standard protocol. The TOPO cloning system is a variation of the TA cloning system allowing the rapid cloning of PCR products, 10 taking advantage from the fact that Taq polymerase leaves a single Adenosine at the 3' end of PCR products. Since the TOPO vector has single-stranded Thymine overhangs, Topoisomerase I enzyme is able to join the T-ends of the vector to the A-overhangs of the PCR product, which can be used without any purification step.

The resulting plasmids (pCRTOPO-ORF series) were used to transform *E. coli* 15 cells (TOP10F', Invitrogen, supplied with the TOPO TA Cloning Kit), obtaining several clones for each ORF. Plasmid DNA was isolated using a commercial kit (WIZARD Plasmid Minipreps; Promega) and sequenced to verify the identity of the amplified and cloned sequence with the originally selected human genomic DNA sequence.

The plasmids containing the desired sequences were used in a further round of 20 PCR reactions necessary for transferring the ORFs into the expression vector pEAK12d (figure 11), which allows the expression of the cloned insert under the control of EF-1 α promoter and in frame with a 6-Histidine Tag sequence, using the Gateway cloning system (Invitrogen).

The expression vector pEAK12D was constructed by modifying pEAK12 (Edge 25 Biosystems). This vector was digested with HindIII and NotI, made blunt ended with

Klenow and dephosphorylated using calf-intestinal alkaline phosphatase. After dephosphorylation, the vector was ligated to blunt ended Gateway reading frame cassette C (Gateway vector conversion system, Invitrogen cat no. 11828-019) that contains AttR recombination sites flanking the ccdB gene (marker for negative selection of non-recombinant plasmids) and chloramphenicol resistance. The resulting plasmids were used to transform DB3.1 *E. coli* cells, which allow propagation of vectors containing the ccdB gene. Miniprep DNA was isolated from several of the resultant colonies and digested with *Asel* / *EcoRI* to identify clones yielding a 670 bp fragment, obtainable only when the cassette had been inserted in the correct orientation. The resultant plasmid was called pEAK12D.

Two series of primers (Table IV) were designed to add the ATT B1 and ATT B2 recombination sites (necessary for the integration in the expression vector) at the 5' and 3' end, respectively, of the ORF-containing insert. In the first series of primers (EX1 series), the original ORF-specific CL primers were modified by adding, at the 5' end, the sequence AAGCAGGCTTCGCCACC (for forward primers) or GTGATGGTGATGGTG (for reverse primers, but after eliminating the nucleotides complementary to the stop codon). In the second series of primers (EX2 series), the original ORF-specific CL primers were modified by adding, at the 5' end, the sequence GGGGACAAGTTGTACAAAAAAGCAGGCTTCGCCACC (for forward primers) or GGGGACCACTTGTACAAGAAAGCTGGGTTCAATGGTGATGGTGATGGTG (for reverse primers, but after eliminating the nucleotides complementary to the stop codon). These reverse primers contain the codons for the 6-Histidine tag, which then results fused in frame with the ORFs at their C-terminal end.

- 39 -

The PCR amplification was performed in 2 consecutive reactions. The first one was performed by mixing the following components (total volume 50 µl in double-distilled water):

25 ng pCRTPOPO-ORF vector
5 mM dNTP (Invitrogen)
0.5 µl Pfx DNA polymerase (Invitrogen)
0.5 µl each EX1 primer (100µM)
5 µl Pfx polymerase buffer 10X (Invitrogen)

The PCR reactions were performed using an initial denaturing step of 95°C for 2 minutes, followed by 10 cycles:

94°C for 15 seconds
68°C for 30 seconds

The PCR products were purified using the Wizard PCR prep DNA purification system (Promega), and added as templates in a second PCR reaction including the following components (total volume 50 µl in double-distilled water):

10 µl purified PCR product
5 mM dNTP (Invitrogen)
0.5 µl Pfx DNA polymerase (Invitrogen)
0.5 µl each EX2 primer (100µM)
5 µl Pfx polymerase buffer 10X (Invitrogen)

The PCR reactions were performed an initial denaturing step of 95°C for 1 minute, followed by 4 cycles:

94°C for 15 seconds
50°C for 30 seconds
68°C for 3 minutes 30 seconds

- 40 -

Then the following conditions were applied for 25 cycles:

94°C for 15 seconds

55°C for 30 seconds

68°C for 3 minutes 30 seconds.

5 The DNA fragments resulting from the PCR reactions were purified as described before and recombined into the pEAK12d vector using the Gateway system.

First, the following 10 µl reactions were assembled:

pDONR-201 (0.1 µg/µl)	1.5 µl
PCR product	5 µl
10 BP buffer	2 µl
BP enzyme mix	1.5 µl

After being incubated at room temperature for 1 hour, the reaction was stopped by adding proteinase K (1 µl, 2 µg) and incubating at 37°C for further 10 minutes.

15 An aliquot of this reaction (2 µl) was used for transforming *E. coli* cells (strain DH10B) by electroporation. Plasmid DNA was prepared for 4 clones for each ORF and used for parallel 10 µl recombination reactions containing:

pEAK12d (0.1 µg / µl)	1.5 µl
Plasmid DNA	1.5 µl
ddH2O	3.5 µl
20 LR buffer	2 µl
LR enzyme mix	1.5 µl

After being incubated at room temperature for 1 hour, the reaction was stopped by adding proteinase K (1 µl, 2 µg) and incubating at 37°C for further 10 minutes. An aliquot of this reaction (1 µl) was used for transforming DH10B *E. coli* cells by 25 electroporation. The clones containing the correct insert were identified first by

performing colony PCR on 3 colonies using the forward and reverse vector primers pEAK12d F1 (GCCAGCTTGGCACTTGATGT) and pEAK12d R1 (GATGGAGGTGGACGTGTCAG), then confirmed by sequencing the insert with the same primer.

5

Example 3: Expression and purification of the 6-Histine-tagged chemokine-like polypeptides in mammalian cells

Human Embryonic Kidney cells expressing the Epstein-Barr virus Nuclear Antigen (HEK293-EBNA) were seeded in T225 flasks (50 ml at a density of 2×10^5 cells/ml) from 16 to 20 hours prior to transfection, which was performed using the cationic polymer reagent JetPEI™ (PolyPlus-transfection; 2 µl/µg of plasmid DNA). For each flask, 113 µg of the ORF-specific pEAK12d plasmid, which were prepared using CsCl (Sambrook, J et al. "Molecular Cloning, a laboratory manual"; 2nd edition. 1989; Cold Spring Harbor Laboratory Press), were co-transfected with 2.3 µg of a plasmid acting as positive control since it expresses Green Fluorescent Protein (GFP). The plasmids, diluted in 230 µl of JetPEI™ solution, were added to 4.6 ml of NaCl 150 mM, vortexed and incubated for 30 minutes at room temperature. This transfection mix was then added to the T225 flask and incubated at 37°C for 6 days. An aliquot of the cultures was then exposed to UV irradiation to check the transfection efficiency by evaluating GFP fluorescence.

Culture medium from HEK293-EBNA cells transfected with the same plasmids were pooled and 100 ml of the medium were diluted to 200 ml with 100 ml of ice -cold buffer A (50 mM NaH₂PO₄; 600 mM NaCl; 8.7 % (w/v) glycerol, pH 7.5), which is the same buffer used for equilibrating the affinity column on which His-tagged proteins were subsequently immobilized and eluted. The solution was filtered through a 0.22 µm

sterile filter (Millipore) and kept at 4°C in 250 ml sterile square media bottles until further processing.

Two consecutive chromatography procedures were applied to the samples at 4°C using an HPLC-based system (Perfusion Chromatography™, PerSeptive Biosystems) including a VISION workstation (BioCAD™ series), POROS™ chromatographic media, and an external 250 ml-sample loader (Labomatic).

In the first chromatography step, a Ni-metal affinity column (0.83 ml, POROS 20 MC) was first regenerated with 30 column volumes of EDTA solution (100 mM EDTA; 1 M NaCl; pH 8.0), and then recharged with Ni ions through washing with 15 column volumes of the Ni solution (100 mM NiSO₄). The column is subsequently washed with 10 column volumes of buffer A, 7 column volumes of buffer B (50 mM NaH₂PO₄; 600 mM NaCl; 8.7 % (w/v) glycerol, 400 mM imidazole, pH 7.5), and finally equilibrated with 15 column volumes of buffer A containing 15 mM imidazole. The sample loader charged the protein-containing solution onto the Ni metal affinity column at a flow rate of 10 ml/min. The column was then washed with 12 column volumes of Buffer A, followed by 28 column volumes of Buffer A containing a concentration of imidazole (20 mM) allowing the elution of contaminating proteins that are loosely attached to the Ni-column. The His-tagged protein is finally eluted with 10 column volumes of Buffer B at a flow rate of 2 ml/min, collecting 1.6 ml fractions.

In the second chromatography step, a gel-filtration column (10 ml G-25 Sephadex) was regenerated with 2 ml of buffer D (137 mM NaCl; 2.7 mM KCl; 1.5 mM KH₂PO₄; 8 mM Na₂HPO₄; 1 M NaCl; pH 7.2), and then equilibrated with 2 column volumes of buffer C (137 mM NaCl; 2.7 mM KCl; 1.5 mM KH₂PO₄; 8 mM Na₂HPO₄; 20 % (w/v) glycerol; pH 7.4) before injecting the Ni-column peak fractions onto this column. The sample is eluted with buffer C and the desalted sample is recovered in 2.2

ml fractions. The peak fractions from the gel-filtration column were filtered through a 0.22 µm sterile centrifugation filter (Millipore) and aliquots (20 µl) were analyzed in parallel on SDS-PAGE (4-12 % NuPAGE gel; Novex) by Coomassie staining and by Western blot with antibodies recognizing Histidine tags. Protein concentrations were 5 determined in the samples that show detectable protein bands by Coomassie staining, using the BCA Protein Assay kit (Pierce) and Bovine Serum Albumin as standard.

The gel for the Western blot analysis was electrotransferred to a nitrocellulose membrane at 290 mA at 4°C for 1 hour. The membrane is blocked with 5 % milk powder in PBS (137 mM NaCl; 2.7 mM KCl; 1.5 mM KH₂PO₄; 8 mM Na₂HPO₄; pH 7.4), 10 and subsequently incubated with a mixture of 2 rabbit polyclonal anti-Histidine tag antibodies (G-18 and H-15, 0.2 µg/ml each; Santa Cruz) at 4°C overnight. After a further 1 hour incubation at room temperature, the membrane was washed with PBS containing 0.1% Tween-20 (3 x 10 min), and then exposed to a secondary HRP-conjugated anti-rabbit antibody (DAKO) at room temperature for 2 hours. After washing 15 in PBS containing 0.1% Tween-20 (3 x 10 minutes), the ECL kit (Amersham Pharmacia) was used to detect the antibodies immobilized onto the membrane, comparing the film with the image of the Coomassie stained gel.

Example 4: Cell- and Animal-based assay for the validation and characterization 20 of the chemokine-like polypeptides.

Several assays have been developed for testing specificity, potency, and efficacy of chemokines using cell cultures or animal models, for example *in vitro* chemotaxis assays (Proudfoot AE et al., 2001; Lusti-Narasimhan M et al., 1995), or mouse ear swelling (Garrigue JL et al., 1994). Many other assays and technologies for 25 generating useful tools and products (antibodies, transgenic animals, radiolabeled

proteins, etc.) have been described in reviews and books dedicated to chemokines (Methods Mol. Biol vol. 138, "Chemokines Protocols", edited by Proudfoot AI et al., Humana Press Inc., 2000; Methods Enzymol, vol. 287 and 288, Academic Press, 1997), and can be used to verify, in a more precise manner, the biological activities of 5 the chemokine-like polypeptides of the invention and related reagents in connection with possible therapeutic or diagnostic methods and uses.

TABLE I

Amino Acid	Synonymous Groups	More Preferred Synonymous Groups
Ser	Gly, Ala, Ser, Thr, Pro	Thr, Ser
Arg	Asn, Lys, Gln, Arg, His	Arg, Lys, His
Leu	Phe, Ile, Val, Leu, Met	Ile, Val, Leu, Met
Pro	Gly, Ala, Ser, Thr, Pro	Pro
Thr	Gly, Ala, Ser, Thr, Pro	Thr, Ser
Ala	Gly, Thr, Pro, Ala, Ser	Gly, Ala
Val	Met, Phe, Ile, Leu, Val	Met, Ile, Val, Leu
Gly	Ala, Thr, Pro, Ser, Gly	Gly, Ala
Ile	Phe, Ile, Val, Leu, Met	Ile, Val, Leu, Met
Phe	Trp, Phe, Tyr	Tyr, Phe
Tyr	Trp, Phe, Tyr	Phe, Tyr
Cys	Ser, Thr, Cys	Cys
His	Asn, Lys, Gln, Arg, His	Arg, Lys, His
Gln	Glu, Asn, Asp, Gln	Asn, Gln
Asn	Glu, Asn, Asp, Gln	Asn, Gln
Lys	Asn, Lys, Gln, Arg, His	Arg, Lys, His
Asp	Glu, Asn, Asp, Gln	Asp, Glu
Glu	Glu, Asn, Asp, Gln	Asp, Glu
Met	Phe, Ile, Val, Leu, Met	Ile, Val, Leu, Met
Trp	Trp, Phe, Tyr	Trp

TABLE II

Amino Acid	Synonymous Groups
Ser	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Arg	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-.Met, D-Ile, Orn, D-Orn
Leu	D-Leu, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Pro	D-Pro, L-L-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Thr	D-Thr, Ser, D-Ser, allo-Thr, Met,D-Met, Met(O), D-Met(O), Val, D-Val
Ala	D-Ala, Gly, Aib, B-Ala, Acp, L-Cys, D-Cys
Val	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met, AdaA, AdaG
Gly	Ala, D-Ala, Pro, D-Pro, Aib, .beta.-Ala, Acp
Ile	D-Ile, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Phe	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, AdaA, AdaG, cis-3,4, or 5-phenylproline, Bpa, D-Bpa
Tyr	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Cys	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Gln	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Asn	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Lys	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Asp	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Glu	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Met	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val

- 47 -

TABLE III

SEQ ID NO:	NAME	DIRECTION	5' ->3' SEQUENCE
17	CL_1754_5	Forward	ATGAATGTCATTGTTTACA
18	CL_1754_3	Reverse	CTACCAACCT GTACAGCATG
19	CL_4922_5	Forward	CTGACTATGATGAGGGTGCT AAGGC
20	CL_4922_3	Reverse	TCAAATTGCTGGGAAAGTTC TCAGG
21	CL_5008_5	Forward	CATGATCTTGGCCTGCTAA TC
22	CL_5008_3	Reverse	TTAAAGGGAA AGTAATAGGA G
23	CL_0210_5	Forward	CTATGGGCTTGTGTTCTA TG
24	CL_0210_3	Reverse	TCAGAAAAATTCTAACAAAA TTG
25	CL_0711_5	Forward	ATGGTTATTCCACATCTTG
26	CL_0711_3	Reverse	TCATCTCTGTTGCAGCAAAC G
27	CL_4320_5	Forward	ATGTTATTTACTTATTATT C
28	CL_4320_3	Reverse	TCACAGAAAA ATCAAAGAGG

TABLE IV

SEQ ID NO:	NAME	DIRECTION	5' - 3' SEQUENCE
29	EX1_1754_5	Forward	AAGCAGGCTTCGCCACC ATGAATGTCATTGTTTACA
30	EX1_1754_3	Reverse	GTGATGGTGATGGTG CCAACCTGTACAGCATG
31	EX1_4922_5	Forward	AAGCAGGCTTCGCCACC CTGACTATGATGAGGGTGCT AAGGC
32	EX1_4922_3	Reverse	GTGATGGTGATGGTG AATTGCTGGGAAAGTTTC TCAGG
33	EX1_5008_5	Forward	AAGCAGGCTTCGCCACC CATGATCTTGGCCTGCTAA TC
34	EX1_5008_3	Reverse	GTGATGGTGATGGTG AAGGGAAAGTAATAGGAG
35	EX1_0210_5	Forward	AAGCAGGCTTCGCCACC CTATGGGCTTGTGTTCTA TG
36	EX1_0210_3	Reverse	GTGATGGTGATGGTG GAAAAATCTAACAAAA TTG
37	EX1_0711_5	Forward	AAGCAGGCTTCGCCACC ATGGTATTCCACATCTTG
38	EX1_0711_3	Reverse	GTGATGGTGATGGTG TCTCTGTTGCAGCAAAC G
39	EX1_4320_5	Forward	AAGCAGGCTTCGCCACC ATGTTATTACTTTATTATT C
40	EX1_4320_3	Reverse	GTGATGGTGATGGTG CAGAAAAATCAAAGAGG
41	EX2_1754_5	Forward	GGGGACAAGTTTGACAAAAAAAGCAGGCTTCGCCACC ATGAATGTC ATTGTTTACA
42	EX2_1754_3	Reverse	GGGGACCAACTTTGACAGAAAGCTGGGTTCAATGGTGATGGTGA TGGTGCCAACCTGTACAGCATG
43	EX2_4922_5	Forward	GGGGACAAGTTTGACAAAAAAAGCAGGCTTCGCCACC CTGACTATG ATGAGGGTGCTAAGGC
44	EX2_4922_3	Reverse	GGGGACCAACTTTGACAGAAAGCTGGGTTCAATGGTGATGGTGA TGGTGAATTGCTGGGAAAGTC TCAGG
45	EX2_5008_5	Forward	GGGGACAAGTTTGACAAAAAAAGCAGGCTTCGCCACC CATGATCTT TGGCTGCTAATC
46	EX2_5008_3	Reverse	GGGGACCAACTTTGACAGAAAGCTGGGTTCAATGGTGATGGTGA TGGTGAAGGGAAAGTAATAGGAG
47	EX2_0210_5	Forward	GGGGACAAGTTTGACAAAAAAAGCAGGCTTCGCCACC CTATGGGCT TTGTTGTTCTATG
48	EX2_0210_3	Reverse	GGGGACCAACTTTGACAGAAAGCTGGGTTCAATGGTGATGGTGA TGGTGGAAAAATTCTAACAAAA TTG
49	EX2_0711_5	Forward	GGGGACAAGTTTGACAAAAAAAGCAGGCTTCGCCACC ATGGTTATT CCACATCTG
50	EX2_0711_3	Reverse	GGGGACCAACTTTGACAGAAAGCTGGGTTCAATGGTGATGGTGA TGGTGCTCTGTTGCAGCAAAC G
51	EX2_4320_5	Forward	GGGGACCAACTTTGACAAAAAAAGCAGGCTTCGCCACC ATGTTATT ACTTTATTATC
52	EX2_4320_3	Reverse	GGGGACCAACTTTGACAGAAAGCTGGGTTCAATGGTGATG GTGA TGGTGCAAGAAAATCAAAGAGG

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CLAIMS

1. An isolated polypeptide having chemotactic activity selected from the group consisting of:
 - a) the amino acid sequences SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16;
 - b) the mature form of the polypeptides SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16;
 - c) the polypeptides comprising the Cysteine-rich region of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16, as indicated in fig. 9 and 10;
 - d) the active variants of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 wherein any amino acid specified in the chosen sequence is non-conservatively substituted, provided that no more than 15% of the amino acid residues in the sequence are so changed;
 - e) the active fragments, precursors, salts, or derivatives of the amino acid sequences given in a) to d).
- 15 2. The polypeptide of claim 1 that is a naturally occurring allelic variant of the sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16.
3. The polypeptide of claim 2, wherein the variant is the translation of a single nucleotide polymorphism.
- 20 4. The polypeptide of any of the claims from 1 to 3, wherein the polypeptide binds specifically an antibody or a binding protein generated against SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 or a fragment thereof.

5. A fusion protein comprising a polypeptide according to any of the claims from 1 to 4.
6. The fusion proteins of claim 6 wherein said proteins further comprise one or more 5 amino acid sequence belonging to these protein sequences: membrane-bound protein, immunoglobulin constant region, multimerization domains, extracellular proteins, signal peptide-containing proteins, export signal-containing proteins.
7. An antagonist of a polypeptide of any of the claims from 1 to 4, wherein said 10 antagonist comprises an amino acid sequence resulting from the modification of one or more residues of said polypeptide.
8. A ligand binding specifically to a polypeptide according to any one of claims 1 to 4.
- 15 9. The ligand of claim 8 that antagonizes or inhibits the chemotactic activity of a polypeptide according to any one of claims 1 to 4.
10. A ligand according to claim 11 which is a monoclonal antibody, a polyclonal 20 antibody, a humanized antibody, an antigen binding fragment, or the extracellular domain of a membrane-bound protein.
11. The polypeptides of any of the claims from 1 to 7 or of claim 10, wherein said 25 polypeptides are in the form of active conjugates or complexes with a molecule chosen amongst radioactive labels, fluorescent labels, biotin, or cytotoxic agents.

12. A peptide mimetic designed on the sequence and/or the structure of a polypeptide according to any one of claims 1 to 4.

5 13. An isolated nucleic acid encoding for an isolated polypeptide selected from the group consisting of:
a) the polypeptides having chemotactic activity of any of the claims from 1 to 4;
b) the fusion proteins of claim 5 or 6; or
c) the antagonists of claim 7.

10

14. The nucleic acid of claim 13, comprising a DNA sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 15, or the complement of said DNA sequences.

15 15. A purified nucleic acid which:

a) hybridizes under high stringency conditions, or
b) exhibits at least about 85% identity over a stretch of at least about 30 nucleotides,

with a nucleic acid selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9,

20 11, 13, or 15, or a complement of said DNA sequences

16. A vector comprising a nucleic acid of any of claims from 13 to 15.

17. The vector of claim 16, wherein said nucleic acid molecule is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic host cells of the encoded polypeptide.

5 18. The polypeptides encoded by the purified nucleic acids of claim 15.

19. A process for producing cells capable of expressing a polypeptide of any the claims from 1 to 7 or of claim 18, comprising genetically engineering cells with a vector or a nucleic acid according to any of the claims from 13 to 17.

10

20. A host cell transformed with a vector or a nucleic acid according to any of the claims from 13 to 17.

15 21. A transgenic animal cell that has been transformed with a vector or a nucleic acid according to any of the claims from 13 to 17, having constitutive or inducible altered expression levels of a polypeptide according to any one of claims from 1 to 4.

20 22. A transgenic non-human animal that has been transformed to have enhanced or reduced expression levels of a polypeptide according to any one of claims from 1 to 4.

23. A method for making a polypeptide of any the claims from 1 to 7 comprising culturing a cell of claim 20 or 21 under conditions in which the nucleic acid or

vector is expressed, and recovering the polypeptide encoded by said nucleic acid or vector from the culture.

24. A compound that enhances the expression level of a polypeptide according to
5 any one of claims from 1 to 4 into a cell or in an animal.
25. A compound that reduces the expression level of a polypeptide according to any
one of claims from 1 to 4 into a cell or in an animal.
- 10 26. The compound of claim 24 that is an antisense oligonucleotide or a small
interfering RNA.
27. Purified preparations containing a polypeptide of any of the claims from 1 to 6 or
claim 18, an antagonist of claim 7, a ligand of any of the claims from 8 to 10,
15 peptide mimetic of claim 12, a nucleic acid of any of the claims from 13 to 17, a
cell of claim 20 or 21, or a compound of any of the claims from 24 to 26.
28. Use of a polypeptide of any of the claims from 1 to 6 or claim 18, a peptide
mimetic of claim 12, a nucleic acid of any of the claims from 13 to 17, a cell of
20 claim 20 or 21, or a compound of claim 24, in the therapy or in the prevention of a
disease when the increase in the chemotactic activity of a polypeptide of any of
the claims from 1 to 4 is needed.
29. Pharmaceutical compositions for the treatment or prevention of diseases needing
25 an increase in the chemotactic activity of a polypeptide of any of the claims from

- 58 -

1 to 6 or claim 18, a peptide mimetic of claim 12, a nucleic acid of any of the claims from 13 to 17, a cell of claim 20 or 21, or a compound of claim 24, as active ingredient.

5 30. Process for the preparation of pharmaceutical compositions, which comprises combining a polypeptide of any of the claims from 1 to 6 or claim 18, a peptide mimetic of claim 12; a nucleic acid of any of the claims from 13 to 17, a cell of claim 20 or 21, or a compound of claim 24, together with a pharmaceutically acceptable carrier.

10

31. Method for the treatment or prevention of diseases needing an increase in the chemotactic activity of a polypeptide of any of the claims from 1 to 4, comprising the administration of a therapeutically effective amount of a polypeptide of any of the claims from 1 to 6 or claim 18, a peptide mimetic of claim 12, a nucleic acid of any of the claims from 13 to 17, a cell of claim 20 or 21, or a compound of claim 24.

15

32. Use of an antagonist of claim 7, a ligand of any of the claims from 8 to 10, or of a compound of claims 25 or 26, in the therapy or in the prevention of a disease associated to the excessive chemotactic activity of a polypeptide of any of the claims from 1 to 4.

20

33. Pharmaceutical compositions for the treatment or prevention of a disease associated to the excessive chemotactic activity of a polypeptide of any of the

claims from 1 to 4, containing an antagonist of claim 7, a ligand of any of the claims from 8 to 10, or of a compound of claims 25 or 26, as active ingredient.

34. Process for the preparation of pharmaceutical compositions for the treatment or prevention of diseases associated to the excessive chemotactic activity of a polypeptide of any of the claims from 1 to 4, which comprises combining an antagonist of claim 7, a ligand of any of the claims from 8 to 10, or of a compound of claims 25 or 26, together with a pharmaceutically acceptable carrier.
- 10 35. A method for the treatment or prevention of diseases related to the polypeptide of any of the claims from 1 to 4, comprising the administration of a therapeutically effective amount of an antagonist of claim 7, a ligand of any of the claims from 8 to 10, or of a compound of claims 25 or 26.
- 15 36. A method for screening candidate compounds effective to treat a disease related to the chemokine-like polypeptides of any of the claims from 1 to 4, comprising:
 - (a) contacting a cell of claim 20, a transgenic animal cell of claim 21, or a transgenic non-human animal according to claim 22, having enhanced or reduced expression levels of the polypeptide, with a candidate compound and
 - 20 (b) determining the effect of the compound on the animal or on the cell.
37. A method for identifying a candidate compound as an antagonist/inhibitor or agonist/activator of a polypeptide of any of the claims 1 to 4 comprising:
 - (a) contacting said polypeptide, said compound, and a mammalian cell or a mammalian cell membrane capable of binding the polypeptide; and

- 60 -

(b) measuring whether the molecule blocks or enhances the interaction of the polypeptide, or the response that results from such interaction, with the mammalian cell or the mammalian cell membrane..

5 38. A method for determining the activity and/or the presence of the polypeptide of any the claims from 1 to 4 in a sample, the method comprising:
(a) providing a protein-containing sample;
(b) contacting said sample with a ligand of any of the claims from 8 to 10; and
(c) determining the presence or said ligand bound to said polypeptide.

10

39. A method for determining the presence or the amount of a transcript or of a nucleic acid encoding the polypeptide of any the claims from 1 to 4 in a sample, the method comprising:
(a) providing a nucleic acids-containing sample;
(b) contacting said sample with a nucleic acid of any of the claims 13 to 17; and
(c) determining the hybridization of said nucleic acid with a nucleic acid into the sample.

20 40. Use of the primer sequences containing the sequences SEQ ID NO: 17 -28 for determining the presence or the amount of a transcript or of a nucleic acid encoding a polypeptide of any the claims from 1 to 4 in a sample by Polymerase Chain Reaction

25 41. A kit for measuring the activity and/or the presence of the chemokine -like polypeptides of any of the claims from 1 to 4 in a sample comprising one or more

- 61 -

of the following reagents: a polypeptide of any of the claims from 1 to 6 or claim
18, an antagonist of claim 7, a ligand of any of the claims from 8 to 10, a
polypeptide of claim 11, a peptide mimetic of claim 12, a nucleic acid of any of
the claims from 13 to 17, a cell of claim 20 or 21, a compound of any of the
claims from 24 to 26, a pharmaceutical composition of claims 29 or 33, or primer
5 sequences containing the sequences SEQ ID NO: 17-28.

1/11

Figure 1

		<u>forward</u>	
GNSQ_1754	gaggta	atg aat gtc att gtt tta cag ttt att ctt ctt gtg ttt ctt ctt	51
p1754		<u>Met Asn Val Ile Val</u> Gln Phe Ile Leu Leu Val Phe Leu Leu	
		S S S S S S S S	15
GNSQ_1754	gtg aag ata tat aag cat	gca gac aca cta ttt tat ata tat ata	96
p1754	<u>Val Lys Ile Tyr Lys His Ala</u>	Asp Thr Leu Phe Tyr Ile Tyr Ile	30
	S		
GNSQ_1754	cct ata tat gta tgg atg tgg atg tgg atg tgg atg tgg atg tgg	ata cac agt tat gca ctg tat aac	141
p1754	Pro Ile Tyr Val Cys Met Cys Ile His Ser Tyr Ala Leu Tyr Asn		45
	S S		
GNSQ_1754	agt att ttg gtc agt gat gga ctg cgt atg cta agg tgg tcc cat	186	
p1754	Ser Ile Leu Val Ser Asp Gly Leu Arg Met Leu Arg Cys Ser His	60	
	S		
GNSQ_1754	aag att ata ata agt act ttg act ata act ttt cta tgg tta cat	231	
p1754	Lys Ile Ile Ile Ser Thr Leu Thr Ile <u>Thr Phe Leu Cys Leu His</u>	75	
	S		
GNSQ_1754	gca gaa ata ctt act aat ggg tta cag ttg cct aca gta ttc agt	276	
p1754	<u>Ala Glu Ile Leu Thr Asn Gly Leu Gln Leu Pro Thr Val Phe Ser</u>	90	
		<u>reverse</u>	
GNSQ_1754	aca cca gca tgc tgg atc ggt tgg tag	303	
p1754	Thr Pro Ala Cys Cys Thr Gly Trp STOP	98	
	S		

2/11

Figure 2

3/11

Figure 3

GNSQ_2882	ggaagt atg agt cct agt tta ttc ttc att ttt aag att gtt ttg gct att	51
p2882	<u>Met Ser Pro Ser Leu Phe Phe Ile Phe Lys Ile Val Leu Ala Ile</u>	15
	S S S S S	
GNSQ_2882	gtg gat tcc ctg caa ttc tat atg aat ttc gaa tca gtt tgt cga	96
p2882	<u>Val Asp Ser Leu Gln Phe Tyr Met Asn Phe Glu Ser Val Cys Arg</u>	30
	S	
GNSQ_2882	tgt ctg caa aaa atc tct gtg att ctg ata ggg att gct ttt aac	141
p2882	<u>Cys Leu Gln Lys Ile Ser Val Ile Ile Leu Ile Gly Ile Ala Phe Asn</u>	45
	S	
GNSQ_2882	ctg tgt aac gat ttg ggg agt att gtc att tta aca gtg tta tgt	186
p2882	<u>Leu Cys Asn Asp Leu Gly Ser Ile Val Ile Leu Thr Val Leu Cys</u>	60
	S	
GNSQ_2882	att cta atc cat gaa tat gaa ata tat ttc ctt ttg ttt aga tct	231
p2882	<u>Ile Leu Ile His Glu Tyr Glu Ile Tyr Phe Leu Leu Phe Arg Ser</u>	75
GNSQ_2882	ttg att ttt tca tta tgt ttt ata gtt cca gag tat agt aag ttt	276
p2882	<u>Leu Ile Phe Ser Leu Cys Phe Ile Val Pro Glu Tyr Ser Lys Phe</u>	90
	S	
GNSQ_2882	tgc aat ttt tat gtt aaa ttt att ctt aag aat tta ttt ttg atg	321
p2882	<u>Cys Asn Phe Tyr Val Lys Phe Ile Leu Lys Asn Leu Phe Leu Met</u>	105
GNSQ_2882	cta tca taa	330
p2882	<u>Leu Ser STOP</u>	107
	S	

4/11

Figure 4

GNSQ_4711	tctagg atg gta act cct atc tgg aca ctt ttc att tgt tac tgt ttg acc	51
p4711	<u>Met Val Thr Pro Ile Trp Thr Leu Phe Ile Cys Tyr Cys Leu Thr</u>	15
	S .	
GNSQ_4711	tct ttg ctt gta tta cag gct ata ttt aaa gaa ata gat aac att	96
p4711	<u>Ser Leu Leu Val Leu Gln Ala</u> Ile Phe Lys Glu Ile Asp Asn Ile	30
	S S S S	
GNSQ_4711	ctc tct gag gtt gat tta aac caa cat cct gta cgt tgc tgc tat	141
p4711	<u>Leu Ser Glu Val Asp Leu Asn Gln His Pro Val Arg Cys Cys Tyr</u>	45
	S S	
GNSQ_4711	agc ttc cca aca ttt tgt gta gag ggg atg cta ttg aag ttg tgt	186
p4711	<u>Ser Phe Pro Thr Phe Cys Val Glu Gly Met Leu Lys Leu Cys</u>	60
GNSQ_4711	ttt aat atg gag cca cac tgt ttt ctt tct ctg acc cag tct aca	231
p4711	<u>Phe Asn Met Glu Pro His Cys Phe Leu Ser Leu Thr Gln Ser Thr</u>	75
	S	
GNSQ_4711	gtc agc ctg tcc caa ggc tgc cat cta ttc tct gtg ttt gtg cag	276
p4711	<u>Val Ser Leu Ser Gln Gly Cys His Leu Phe Ser Val Phe Val Gln</u>	90
	S	
GNSQ_4711	ctc atc tgg aca gct cat ctg gac aga cac aaa gaa tag	315
p4711	<u>Leu Ile Trp Thr Ala His Leu Asp Arg His Lys Glu STOP</u>	102
	S	

5/11

Figure 5

		forward	
GNSQ_4320	tgtaaat	atg tta ttt act tta tta ttc cga att cta atc ggt tat gtg aga	51
p4320		Met Leu Phe Thr Leu Leu Phe Arg Ile Leu Ile Gly Tyr Val Arg	15
		\$ \$ \$ \$ \$ \$ \$	
GNSQ_4320		act ctg tgg acg aaa aat tct tgc tgc tgt tgg cga atg att tta	96
p4320		Thr Leu Trp Thr Lys Asn Ser Cys Cys Cys Trp Arg Met Ile Leu	30
		\$ \$	
GNSQ_4320		aat cat tca ttt aaa caa gaa gtg cct atg att gta gag cta aag	141
p4320		Asn His Ser Phe Lys Gln Glu Val Pro Met Ile Val Glu Leu Lys	45
GNSQ_4320		caa aaa tgt gaa atg ttt tgt cag aaa tat cta gtt gat aaa gat	186
p4320		Gln Lys Cys Glu Met Phe Cys Gln Lys Tyr Leu Val Asp Lys Asp	60
		\$	
GNSQ_4320		tat tcc ttt cgt gtt tct gta acc tgt cag ttc ttt ata ctt tta	231
p4320		Tyr Ser Phe Arg Val Ser Val Thr Cys Gln Phe Phe Ile Leu Leu	75
		\$	
GNSQ_4320		cat gat tcc tac cca act gag aat aca tgg tca act att cca aca	276
p4320		His Asp Ser Tyr Pro Thr Glu Asn Thr Trp Ser Thr Ile Pro Thr	90
		\$	
		reverse	
GNSQ_4320		ttg tct gct ctt ata tcc tct ttg att ttt ctg tga	312
p4320		Leu Ser Ala Leu Ile Ser Ser Leu Ile Phe Leu STOP	101
		\$	

6/11

Figure 6

		forward	
GNSQ_5008	ccagac	atg atc ttt ggc ctg cta atc aaa gct ctt tat cta gcg tca gcc	51
p5008		Met Ile Phe Gly Leu Leu Ile Lys Ala Leu Tyr Leu Ala Ser Ala	
		S S S S	15
GNSQ_5008	tgg gca ggg gct ctg agc ctc ggc gct gct ggc att tgg ggc tgg	96	
p5008	Trp Ala Gly Ala Leu Ser Leu Gly Ala Ala Gly Ile Trp Gly Trp	30	
GNSQ_5008	atg act ctt tgc tgt ggc tgc tgt cct gtg cat tac agg aca tta	141	
p5008	Met Thr Leu Cys Cys Gly Cys Cys Pro Val His Tyr Arg Thr Leu	45	
	S S		
GNSQ_5008	cgt agc atc cct gac cac aac cta cta gat gcc agt agc acc ccc	186	
p5008	Arg Ser Ile Pro Asp His Asn Leu Leu Asp Ala Ser Ser Thr Pro	60	
GNSQ_5008	tcc cta gtt atg aca acc aga aac atc tcc aga cat tgc caa tgt	231	
p5008	Ser Leu Val Met Thr Arg Asn Ile Ser Arg His Cys Gln Cys	75	
	S		
GNSQ_5008	ccc ctg gtg gca aaa tca tcc ccg gct gag aat gag tgt tgc acg	276	
p5008	Pro Leu Val Ala Lys Ser Ser Pro Ala Glu Asn Glu Cys Cys Thr	90	
	S		
GNSQ_5008	gta att cct cca ttc caa att aac aga gca ctt agg aac gag tgc	321	
p5008	Val Ile Pro Pro Phe Gln Ile Asn Arg Ala Leu Arg Asn Glu Cys	105	
		reverse	
GNSQ_5008	ttt ctc cta tta ctt tcc ctt taa	345	
p5008	Phe Leu Leu Leu Ser Leu STOP	112	
	S		

7/11

Figure 7

		forward	
GNSQ_0210	tgaact	atg ggc ttt gtt cta tgc cta att ttc ttc ctg tgt aag act	51
p0210		Met Gly Phe Val Val Cys Leu Ile Phe Leu Cys Lys Thr	15
		\$ \$ \$ \$ \$	
GNSQ_0210	gga atg gat tcc aga ttt caa cta aaa ctc ttg ttt cac tgt ttt	96	
p0210	Gly Met Asp Ser Arg Phe Gln Leu Lys Leu Leu Phe His Cys Phe	30	
GNSQ_0210	caa gga ctt ttc caa agg tca cac atg gac tat tgt gat gaa tgc	141	
p0210	Gln Gly Leu Phe Gln Arg Ser His Met Asp Tyr Cys Asp Glu Cys	45	
	\$ \$		
GNSQ_0210	act ctg cag ggt gtg ttc cca gag cac aga agt aac cag aga gct	186	
p0210	Thr Leu Gln Gly Val Phe Pro Glu His Arg Ser Asn Gln Arg Ala	60	
GNSQ_0210	gca agg gag gtg ttg ccc aca cca aaa cac tgc aga ctt att ccc	231	
p0210	Ala Arg Glu Val Leu Pro Thr Pro Lys His Cys Arg Leu Ile Pro	75	
	\$		
GNSQ_0210	ctg ggg aca gtg ctg tca gag tgt cca ttt caa gct ccc tgt tgg	276	
p0210	Leu Gly Thr Val Leu Ser Glu Cys Pro Phe Gln Ala Pro Cys Trp	90	
	\$		
GNSQ_0210	cca cag aca aaa gcc att atc cta aat ctc tgg cga aac ttg gag	321	
p0210	Pro Gln Thr <u>Lys</u> Ala Ile Leu Asn Leu Trp Arg Asn Leu Glu	105	
GNSQ_0210	gtc tta gaa gtg gac aga agt tta aga cag gat tgc ttt aaa tgc	366	
p0210	Val Leu Glu Val Asp Arg Ser Leu Arg Gln Asp Cys Phe Lys Cys	120	
	← reverse		
GNSQ_0210	aca att ttg tta gaa ttt ttc tga	390	
p0210	Thr Ile Leu Glu Phe Phe STOP	127	
	\$		

8/11

Figure 8

		forward	
GNSQ_4922	ctgact	atg atg agg gtg cta agg	51
p4922		ctg ctg qcq agg gtc ctc ctc ggc cag	
		Met Met Arg Val Leu Arg Leu Ala Arg Val Leu Leu Gly Gln	15
		S S S S S S	
GNSQ_4922		ctt ctc cta gca gca ggg cac gca cag ccc tgt ttt ctc atc tgc	96
p4922		Leu Leu Leu Ala Ala Gly His Ala Gln Pro Cys Phe Leu Ile Cys	30
		S S	
GNSQ_4922		ttt cag cag cat ttg cct cct act cca ctt ggg tca ctc aag ggt	141
p4922		Phe Gln Gln His Leu Pro Pro Thr Pro Leu Gly Ser Leu Lys Gly	45
GNSQ_4922		ccc aaa ata gac ctg tgc att cat ggg acc cct ccc acc tgc ctc	186
p4922		Pro Lys Ile Asp Leu Cys Ile His Gly Thr Pro Pro Thr Cys Leu	60
		S	
GNSQ_4922		tct gct cag tgt ctc tgt tgg gac agg cag caa gtg ctt aaa tcc	231
p4922		Ser Ala Gln Cys Leu Cys Trp Asp Arg Gln Gln Val Leu Lys Ser	75
		S	
GNSQ_4922		← cag cca ctg ctc ccc gct gga gtc cac ctg aga act ttc cca gca	276
p4922		Gln Pro Leu Leu Pro Ala Gly Val His Leu Arg Thr Phe Pro Ala	90
		←	
GNSQ_4922		att tga	282
p4922		Ile STOP	91
		S	

9/11

Figure 9N-Terminal Region

CXCL1	MARAALS --AAPSNPR ---LLRVALLLLLLVAAGRRAAG
CXCL2	MARATLS --AAPSNPR ---LLRVALLLLLLVAASRRRAAG
CXCL3	MAHATLS --AAPSNPR ---LLRVALLLLLLVG SRRAGAS
CXCL4	MS --SAF --CASRPG ---LLFLGLLLPLVVAFASA
CXCL5	MSLLSSR --AARVPGPSSSLCALLVLLLLTQPGPIASA
CXCL6	MSLPSSR --AARVPGPGSGSLCALLLLLLTPPGPLASA
CXCL7	MSLRLDTTPSCNSARPLHALQVLLLS1LLTALASSTKGQTKRNLAKGKEE
CXCL8	MT ---SKL-A-----V-ALLAAFLI-SAALCEG
CXCL9	MKKSG -----VLFLLGIIILVLIIGVQG
CXCL10	MNQTA -----I-LICCLIFLTLSGIQC
CXCL11	MSVKGM -----AIALAVILCATVVQG
p1754	MNVI -----VLQFILLVFLLVKTYKHADTLFYI
p0711	MVIPH -----LV-LLTILISFRIKEKNSVFH
p2882	MSPS -----L-FFIFKIVIAIVDSL
p0210	MGFVVLLCLIFFLCKTGMDSRFQLKLLFHCFQGL
p4922	MMR -----VLRLLARVLLGQLLLAA

Cys-rich region

CXCL1	ASVATELRCQ --CLQTLQGIHPKKN -IQSVNVKSPG ----- PHCAQTE --VIATLKNRKA ---C
CXCL2	APLATELRCQ --CLQTLQGIHLKN -IQSVVKVSPG ----- PHCAQTE --VIATLNGOKA ---C
CXCL3	VVTELRCQ --CLQTLQGIHLKN -IQSVNVRSPG ----- PHCAQTE --VIATLKNKKA ---C
CXCL4	EAEEEDGDLQCL --CVKTTSQVPRH -ITSLEVIKAG ----- PHCPTAQ --LIATLKNRKR ---IC
CXCL5	GPAAAVLRELRCV --CLQTTQGVHPKM -ISNLQVFAIG ----- PQCSKVE --VVASLKNKGKE ---IC
CXCL6	GPVSAVLTELR --CLRVTLRVNPKT -IGKLQVFPAG ----- PQCSKVE --VVASLKNKGKQ ---VC
CXCL7	SLDSDLYAELRCM --CIKTTSGIHPKKN -IQSLEVIGKG ----- THCNQVE --VIATLKDRK ---IC
CXCL8	AVLPRSAKELRCQ --CIKTYSKPFHPKFIKELRVIESG ----- PHCANTE --IIVKLSDGRE ---LC
CXCL9	TPVVRKGRC --CISTNQGTTIHLQSLKDLKQFAPS ----- PSCEKIE --IIATLNGVQT ---C
CXCL10	VPLSRTVRC --CISISNQPVNPRSLEKLEIIPAS ----- QFCPRVE --IIATMKKKGEKR ---C
CXCL11	FPMFKRGRCL --CIGPGVKAVKVADIEKASIMYPS ----- NNCDKIE --VIITLKENKGQR ---C
p1754	YIPIYVCM --C1H-SYALYNNSILVSDGLRMLR ----- CSHK --IIISTLTITF ---LC
p0711	LIFPAIHSLCL --CDSGRIPARNALDPSDQQPLQQDKDGTETMCVAGSNLNHWSWVNEERK ---C
p2882	QFYMNFESEVCR --CLOKISVIL -IGIAFNLCNDLGS IVILTV-LCILIHVEIYFLLFRSLIFSLC
p0210	FQRSHMDYCDE --CTLQGVFPEHRSNQRAAREVLPPT ----- KHCRLIPLGTVLSECPFQAP ---C
p4922	GHAQPCFLICFQQHLPPTPLGSLKGPKID ----- LCIHGTPTCLSAQC ---LC
	S S S S

C-terminal region

CXCL1	LNPASPIVKKIIEKMLNSDKSN
CXCL2	LNPASPMVKKIIEKMLKNGKSN
CXCL3	LNPASPMVQKIIIEKILNKGSTN
CXCL4	LDLQAPPLYKKIIKKLLES
CXCL5	LDPEAPFLKKVIQKILDGGNKEN
CXCL6	LDPEAPFLKKVIQKILDGSNKKN
CXCL7	LDPDAPRIKKIVQKKLAGDESAD
CXCL8	LDPKENWQRVVEKFLKRAENS
CXCL9	LNPDSADVKELIKKEWQVSKQKKKNGKKHQKKVVLKVRKSQRSRQKKTT
CXCL10	LNPESKAIKNLLKAVSKERSK RSP
CXCL11	LNPKSKQARLIIKKVERKNF
p1754	LHAEILTNGLQPLPTVFSTPACCTGW
p0711	GISIQCNIIQPLQSRKILCRLLQQR
p2882	FIVPEYSKFCNFYVKFILKNLFLMLS
p0210	WPQTKAIILNLWRNLEVLEVDRSLRQDCFKCTILLEFF
p4922	WDRQQVLKSQPLLPAGVHRTFPAl

10/11

Figure 10N-Terminal Region

CCL1	MQIITTAALVCLLLAGMWPEDVD SKSMQV
CCL2	MKVSAALLCLLLIAATFIPQGLAQPDAIN
CCL3	MQVSTAALAVLLCTMALCNQFSASLAAD
CCL4	MKLCVTVLSLLMLVAACFSPALSAPMGSD
CCL5	MKVSAALAVLLIATACAPASASPYSS
CCL7	MKASAALLCLLLTAAAFSPQGLAQPVGIN
CCL8	MKVSAALLCLLLMAATFSPQGLAQPDVS
p4711	MVTPIWTIFICYCLTSLLVLQAFKEIDNILSEVDLNQ
p4320	MLFTLLFRILIGYVRTLW
p5008	MIFGLLIKALYLASAWAGALSLGAAGIWG

Cys-rich region

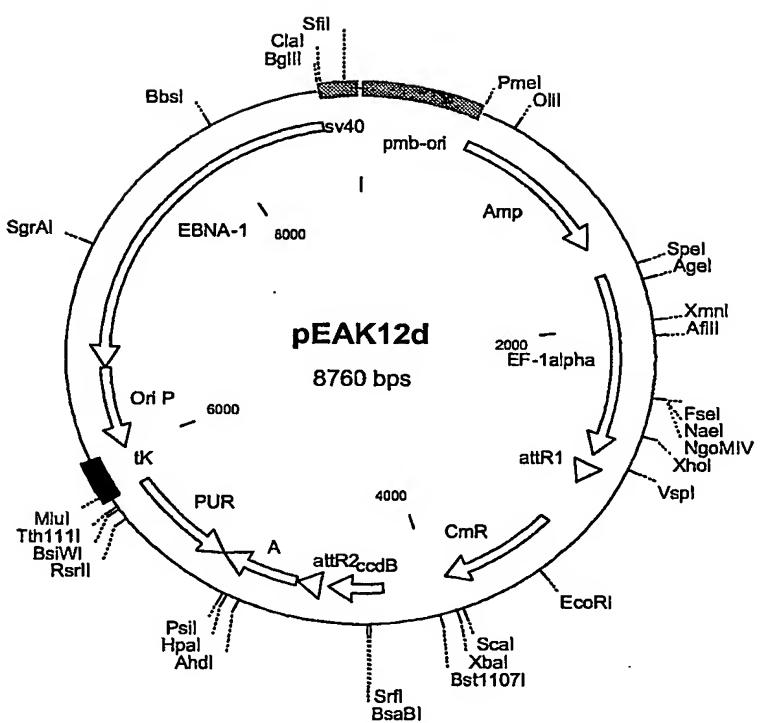
CCL1	PFSSRCCFSFAEQEIPLRAILCYRN -TSSI-----	CSNEGLIPKLKRGKEA -CALD
CCL2	APVTCCYNFTNRKISVQRLLASYRITSSK -----	CPKEAVIFKTTIVAKEI -CAD
CCL3	PTAACCFSYTSRQIPQNFIAFYE -TSSQ-----	CSKPGVIFLTKRSRQV -CAD
CCL4	PTAACCFSYTARKLPRNFFVVDYYE -TSSL-----	CSQPAVVFQTFRSKQV -CAD
CCL5	DTTPCCFAYTARPLPRAHIKEYFY -TSK-----	CSNPAAVVFVTRKRNQV -CAN
CCL7	TSTTCCYRFINKKIPKQRLESYRRTTSSH -----	CPREAVIFKTKLDKEI -CAD
CCL8	IPITCCFNVINRKIPIQRLESYTRITNIQ -----	CPKEAVIFKTKRGKEV -CAD
p4711	HPVRCCYSFPTFCVEGMLLKLCFNMEPH -----	CFLSLTQSTVSLSQG --CHL
p4320	TKNSCCCWRLMILNHSFKQEVPMIVELKQK -----	CEMFQKYLVDKDYSFRVSVTCQFF
p5008	WMTLCCGCCPVHYRTLRSIPDHNLDDASSTPSLVMTRNISRHCQCPLVAKSSPAENE	--CCTV
	SS	S
		S

C-terminal region

CCL1	TVGWVQRHRKMLRHCPSKRK
CCL2	PKQKWKVQDSMDHLDKQTQTPKT
CCL3	PSEEWVQKYVSDLELSA
CCL4	PSESWVQEYVYDLELN
CCL5	PEKKWKVREYINSLEMS
CCL7	PTQKWWQDFMKHLDKKTQTPKL
CCL8	PKERWVRDMSMKHLDQIFONLKP
p4711	FSVVFVQLIWTAAHLDRHKE
p4320	ILLHDSYPTENTWSTIPTLSALISSLIFL
p5008	IPPFQINRALRNECFLLLLSL

11/11

Figure 11



SEQUENCE LISTING

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<130> WO582

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01 APR 2005



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(71) Applicant (for all designated States except US): APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. [NL/NL]; Pietermaai 15, Curacao (AN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): IBBERSON, Mark [GB/CH]; Chemin Planet 41, CH-1188 Gimel (CH). POWER, Christine [GB/FR]; Rue des Jonquilles 10, F-01710 Thoiry (FR). FRAUENSCHUH, Achim [DE/CH]; Chemin de Voirets 1, CH-1228 Plan-les-Ouates (CH).

(74) Agent: SERONO INTERNATIONAL SA INTELLECTUAL PROPERTY; Chemin des Aulx 12, CH-1228 Plan-les-Ouates (CH).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

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Previous Correction:
see PCT Gazette No. 25/2004 of 17 June 2004, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/031233 A3

(54) Title: NOVEL CHEMOKINE-LIKE POLYPEPTIDES

(57) Abstract: The present invention discloses open reading frames (ORFs) in human genome encoding for novel chemokine-like polypeptides, and reagents related thereto including variants, mutants and fragments of said polypeptides, as well as ligands and antagonists directed against them. The invention provides methods for identifying and making these molecules, for preparing pharmaceutical compositions containing them, and for using them in the diagnosis, prevention and treatment of diseases.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 50668

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/52 C12N15/19 A61K38/19 A01K67/027 G01N33/53
C12Q1/68 A61K31/7088 C12N15/62 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL, BIOSIS, PAJ, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE EMBL 'Online! HTG; DNA Human 124964bp 7 March 2000 (2000-03-07), BATES K.: "human DNA sequence from clone RP11-428G23 on chromosome 13" XP002231616 retrieved from EBI Database accession no. AL159154 the whole document -----	1-41
A	DATABASE EMBL 'Online! 26 June 2001 (2001-06-26), OTA T ET AL.: "Human protein sequence SEQ.ID N 12610" XP002231850 retrieved from EBI Database accession no. AAB93409 the whole document -/-	1-41

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the International search

24 March 2004

Date of mailing of the International search report

19.08.04

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Perez, C

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 2001/50668

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>& EP 1 074 617 A (HELIX RES INST) 7 February 2001 (2001-02-07) SEQ:ID n 12610</p> <p>-----</p> <p>HAN WENLING ET AL: "Molecular cloning and characterization of chemokine-like factor 1 (CKLF1), a novel human cytokine with unique structure and potential chemotactic activity."</p> <p>BIOCHEMICAL JOURNAL, vol. 357, no. 1, 2001, pages 127-135, XP002231615 ISSN: 0264-6021 the whole document</p> <p>-----</p>	1-41
A	<p>WELLS T N C ET AL: "THE CHEMOKINE INFORMATION SOURCE: IDENTIFICATION AND CHARACTERIZATION OF NOVEL CHEMOKINES USING THE WORLDWIDEWEB AND EXPRESSED SEQUENCE TAG DATABASES"</p> <p>JOURNAL OF LEUKOCYTE BIOLOGY, FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL, US, vol. 61, May 1997 (1997-05), pages 545-550, XP002925964 ISSN: 0741-5400 cited in the application the whole document</p> <p>-----</p>	1-41
A	<p>BAGGIOLINI M: "CHEMOKINES IN PATHOLOGY AND MEDICINE"</p> <p>JOURNAL OF INTERNAL MEDICINE, OXFORD, GB, vol. 250, no. 2, August 2001 (2001-08), pages 91-104, XP001021499 ISSN: 0954-6820 cited in the application the whole document</p> <p>-----</p>	1-41

INTERNATIONAL SEARCH REPORT

Int'l Application No.
PCT/EP 03/50568

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 31 and 35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: 7-11, 13, 24-25, 27-35, 39-41 searched incompletely because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-41 (partially)

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 31 and 35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 7-11, 13, 24-25, 27-35, 39-41 searched incompletely

Present claims 7-11, 13, 24-25, 27-35, 39, 41 relate to a product/compound defined by reference to a desirable characteristic or a property namely a ligand, an antagonist or an agonist. The claims cover all products/compounds having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for only a very limited number of such products/compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). An attempt is made to define the product/compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to antibodies, peptide mimetics (claims 12), antisenses and small interfering RNAs (claim 26).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/EP 03/50668

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